Food Research

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ION-EXCHANGE CHROMATOGRAPHY OF AMINO ACIDS IN TOMATO JUICE

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(Manuscript received December 24, 1957)

Amino acids are important constituents in food products because of their nutritive value for protein synthesis, their influence on flavor acceptance, and their effect on the storage stability of processed foods *(2)*, especially when the product is stored at higher temperatures *(4)*. Many of the earlier chemical methods for the determination of amino acids were not satisfactory when applied to food materials containing carbohydrates and other substances. Chemical methods based on isolation procedures were frequently laborious and impractical. Lyman and Kuiken *(5)* used microbiological assay procedures to determine amino acid composition of meat. Ion-exchange column chromatography for the quantitative determination of amino acids has been successfully used by Hulme *(3)*, Miller and Johnson *(6)*, Moore and Stein *(7, 8)*, Partridge *(10)*, and many others. If the conditions are carefully followed, successful separation of each individual amino acid is assured.

Lyman and Kuiken *(5)* reported a few essential amino acids in tomatoes, but their work was based only on microbiological methods. Furthermore, no information was given regarding the relative quantities of free and conjugated amino acids in tomatoes. In this report, the nature and quantity of each individual amino acid present in canned tomato juice in both the free and conjugated form were investigated by paper chromatography and by ion-exchange column chromatography method. The effect of nitrogen fertilizer application on yield per acre and on the amino acid content of tomato juice is also reported.

EXPERIMENTAL MATERIALS AND METHODS

Tomato fruits. In a preliminary experiment in 1955 tomatoes of the improved Pearson variety, grown in Alameda County, were processed and analyzed for amino acid distribution in both free and combined form (Table 3). In 1956, the same variety was grown under various levels of fertilizer application on Livermore clay loam soil in a commercial field in Alameda County. The field had been used for growing alfalfa in the previous 4 years and no fertilizers had been applied. For the 1956 season, the tomato plants were transplanted in the field on May 11, and the fertilizers were applied on May 15-16, 6 inches away from the plants and 6 inches deep. Each plot consisted of 4 rows 93 feet long. The plants were set 18 inches apart, and the distance between rows was 5 feet. Nitrogen, phosphorus and potassium were applied in various combinations and amounts to eight plots. The fertilizer treatments are described in Table 1.

The tomatoes were harvested at canning maturity on September 19, 1956, and care was taken to obtain representative samples of uniform maturity as judged by visual skin color.

Canning. Essentially the same procedure was followed in preparing tomato juice from both the 1955 and 1956 packs, with one exception: the 1955 pack was prepared according to the "cold break" process, i.e., the tomatoes were crushed at room temperature, whereas the "hot break" method was used for the 1956 pack. All the processing equipment was made of stainless steel to reduce vitamin C losses.

Each lot consisted of 120 lbs. of tomatoes. The tomatoes were washed with sprays of

* Home address: New Tiryns, Nauplion, Greece.

TABLE 1

Effect of fertilizer treatments on yield and composition of tomatoes
Alameda County, 1956 season

Treatment No.	Fertilizer material	Nutrients added lbs. per acre			Yield [2] tons per acre	° Brix at 20° C.	Total nitrogen mg/100 ml.[3]
		N	P_2O_5	K_2O			
1.....	Control	0	0	0	22.7	5.69	96.43
2.....	NH_4NO_3	120	0	0	29.2	5.61	108.30
3.....	NH_4NO_3 TSP [1]	120	120	0	33.7	5.57	107.90
4.....	NH_4NO_3 TSP [1] K_2SO_4	120	120	120	33.3	5.60	109.40
5.....	NH_3	60	0	0	22.8	5.67	104.50
6.....	NH_3	120	0	0	27.3	5.64	107.40
7.....	NH_3 TSP [1]	120	120	0	33.3	5.47	105.00
8.....	NH_3 TSP [1]	60	60	0	26.9	5.57	103.60

[1] TSP = Triple superphosphate ($CaHPO_4$)
[2] Least significant difference at 5% probability level = 2.55
[3] Least significant difference at 5% probability level = 3.27

cold water, sorted, trimmed, and stemmed. They were then crushed in a hammer mill type "spike-pump" with simultaneous injection of steam at 30 p.s.i.g. ("hot break" process). The temperature of the crushed tomatoes on leaving the spike-pump was 185 ± 5° F. The macerate was pumped into a paddle finisher with a screen of 0.033-inch mesh. The extracted juice was pumped immediately into a high-speed centrifugal vacuum deaerator, operating under 20 inches of vacuum. The deaerated juice was pumped by a positive displacement pump into a steam-heated coil heat exchanger and heated to 198° F. for 15 seconds. The hot juice was pumped into a surge tank, where 0.75 percent NaCl was added, and then filled into No. 2½ cans (401 x 411) at 160° F. The cans were sealed under atmospheric pressure, processed in a rotary cooker at 210° F. for 30 minutes, and cooled in a rotary cooler to 100° F. The samples were stored at 68° F. for 3 to 4 months before the analyses were made. The samples of the 1955 season had been stored at room temperature for 4 months, lyophilized in a Stokes freeze-drier, and kept in sealed jars under refrigeration. When needed, the lyophilized product was reconstructed with distilled water to the same concentration as the fresh tomato juice.

Soluble solids. Soluble solids content was determined by reading the Brix scale on a Zeiss Opton Refractometer. The readings were corrected to that at 20° C. and expressed as percent soluble solids.

Total nitrogen. The standard Kjeldahl method was used. Ten-ml. portions of samples were used for digestion. Fifty ml. of 3% boric acid were used in the Erlenmeyer flasks to receive the distilling ammonia. The results were expressed as mg. nitrogen per 100 ml. of juice.

Qualitative analysis of amino acids. Principally, the free amino acids of the various samples of tomato juice were determined. The two-dimensional paper chromatographic method as described by Block *(1)* was used. The amino acids of each sample were purified by the following procedure: the tomato juice was centrifuged and 10 ml. of the clear serum was applied to a Dowex 50 x 4 cation exchange column. The resin was prepared in the hydrogen form by treating the sodium salt with 2N HCl according to Moore and Stein *(7, 8)*. The column was washed with 150 ml. of distilled water to remove the sugars. The amino acids were liberated by treating the resin with 2N NH_4OH. The solution containing the amino acids was evaporated under vacuum to almost dryness. The residue was dissolved in 10 ml. of distilled water and used directly for paper chromatography. When large amounts of cations were present, the solution of amino acids was

applied to an anion-exchange column (Amberlite in the OH^- form). The cations were washed out with 150 ml. of distilled water, and the amino acids were liberated by treating the resin with an excess of 2N HCl. The solution was evaporated under vacuum to a small volume and then dissolved in distilled water to make 10 ml.

Paper chromatography. Five μl. of the amino acid solution was applied with a micropipette to a Whatman No. 1 (22" x 18") paper. For the two-dimensional paper chromatography, the first solvent was a mixture of freshly distilled phenol and water (80:20 by volume). The phenol was distilled under vacuum in the presence of Zn to avoid oxidation; a beaker containing 50 mg. of KCN in 5 ml. of water was placed in the chromatographic chamber. After 36 hours, the papers were taken out and dried under a ventilated hood. The top layer of a mixture of butanol, acetic acid, and water (4:1:5 by volume) was used as the second solvent and was run at right angles to the first solvent. A beaker containing the same solvent was placed in the chamber. The papers were finally dried as before and sprayed with a solution of 0.3% ninhydrin in 95% ethyl alcohol. The sprayed papers were heated in an electric oven at 180° F. for 10 minutes to develop the color. The amino acids were identified by comparing the R_f values of known amino acids *(1)* and by chromatographing at the same time known amino acids on separate sheets which gave more intense color to the spots examined.

In order to determine the combined amino acids, an acid hydrolysis was carried out by refluxing 5 ml. of the sample with 50 ml. of 6N HCl at the boiling point for 24 hours. The hydrolyzate was filtered and the HCl was removed by evaporation under vacuum. The amino acids were purified by an ion-exchange column, as described above.

In an attempt to identify tryptophane, an alkaline hydrolysis was tried as this amino acid is known to be destroyed during acid hydrolysis. The barium hydroxide method was used *(1)*. Five ml. of tomato juice was mixed with 10 ml. of 14% $Ba(OH)_2$ and the mixture was boiled under a reflux condenser for 20 hours. After hydrolysis the liquid was filtered off and the precipitate was washed with distilled water. The barium ion in the filtrate was precipitated with an excess of 1N H_2SO_4. The $BaSO_4$ was filtered off and washed on the filter paper with hot water. The resulting filtrate was passed through a cation-exchange column for purification of amino acids as described above.

Quantitative analysis of amino acids. The ion-exchange chromatographic method as described by Moore and Stein *(7)* was used. A Dowex 50 x 4 cation-exchange resin (200-mesh) was used. This is a sulfonated polystyrene cross-linked with 4 percent divinyl benzene. Three hundred grams of the resin was treated with 4N HCl on a Buchner funnel until the filtrate became colorless. The resin was washed with water and converted to the sodium form by washing with 2N NaOH until the filtrate became alkaline. The sodium salt was suspended in 3 times its volume of N NaOH and heated over a steam bath for 3 hours with occasional stirring. The alkali treatment was repeated 5 times. The resin was washed free of alkali, passed through a 150-mesh screen with the aid of water, and packed in a water-jacketed column 150 cm. high and with an internal diameter of 0.9 cm. The temperature of the column was controlled by circulating water with a pump from a water bath, which was maintained at a constant temperature by a thermostat. The effluents were received in an automatic constant-volume collector. The buffers were placed in a separatory funnel which was connected to the top of the column by tygon tubing. The flow rate of the buffers was kept at 8 to 10 ml. per hour, the rate being controlled by the level of the buffer in the funnel. Two buffers at pH 3.1 and 5.1, as described by Moore and Stein *(7)*, were used.

Before starting the analysis the temperature of the column was adjusted to 30° C. and 250 ml. of the buffer at pH 3.1 was passed through the column. Two ml. of the tomato juice serum was pipetted into the column and the flow of the buffer (pH 3.1) was started.

Aspartic acid, the first amino acid eluted, was detected after 150 ml. of buffer had run through the column. When the volume of the effluent reached 220 ml., the temperature was raised to 50° C. After the appearance of glutamic acid (350 ml.) the buffer at pH 5.1 was used. The analysis was stopped after the appearance of leucine. A total volume of approximately 500 ml. was collected in each analysis. Figures 1 and 2 show two chromatograms of amino acids on the ion-exchange column used. Either 5-ml. or 3-ml. portions of effluents were collected in the respective volumetric siphons.

The modified ninhydrin-hydrindatin method *(8)* was used for analyzing the effluents. Aliquots of 2 ml. of effluent were pipetted into 10-ml. glass tubes equipped with screw caps. One ml. of the ninhydrin reagent was added, and the tubes were capped, mixed, and heated

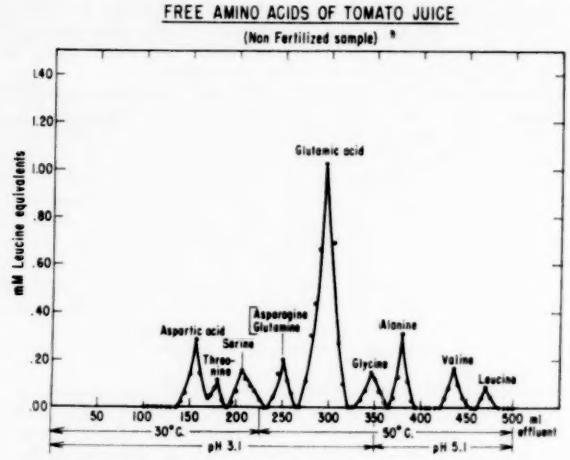

Figure 1. Ion-exchange chromatography for amino acids in tomato juice (non-fertilized sample).

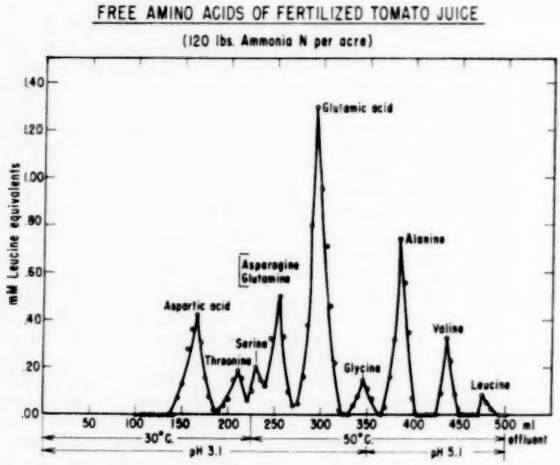

Figure 2. Ion-exchange chromatography for amino acids in tomato juice (120 lbs. ammonia N per acre).

in a boiling water bath for 30 minutes. After cooling to room temperature, the tubes were shaken for 1 minute and diluted with 3 ml. of 50% ethyl alcohol. The blue color was read in a Klett-Summerson photoelectric colorimeter using a green filter (No. 54). A standard curve with various concentrations of leucine was prepared. The color coefficients for the individual amino acids as described by Moore and Stein *(8)* were used. For the identification of the amino acids in the effluents, paper chromatograms were made using small amounts of the effluents where the peaks appeared. The sequence of amino acids in the chromatograms was influenced by the flow rate, the strength of the buffers, and the temperature of

the column. It was necessary, therefore, to keep the whole system under constant and uniform operating conditions.

A synthetic mixture of the amino acids known to be present in tomato juice was chromatographed to assure the position and purity of fractions at each peak.

The amount of each amino acid was calculated by the analytical integration method. The absorbance of the sample (Klett value) was converted to mM of leucine by means of a standard curve. The sum of the leucine milli-equivalents for each amino acid was calculated. The amount of each amino acid present in the effluents is given by the formula:

$$x = \frac{a \times L \times M \times 100}{b \times C \times 1000} \text{ (mg. per 100 ml. tomato juice)}$$

where a = ml. of the effluent fraction (5 or 3 ml.)
b = ml. of the sample used (2 ml.)
C = color coefficient of the amino acid (leucine, C = 1)
L = sum of the leucine mM
M = molecular weight of the amino acid

RESULTS

Effect of fertilizer application on yield and composition. Table 1 shows the effect of various fertilizer treatments on yield and total nitrogen content. The soluble solids content of the tomatoes was not materially changed by the various inorganic plant nutrients, provided the fresh fruit was harvested at comparable stages of maturity.

Paper chromatography of amino acids. The canned tomato juice contained the following free amino acids as determined by two-dimensional paper chromatography: alanine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, leucine, serine, threonine, and valine. There was no detectable change in the qualitative amino acid composition due to the application of fertilizers. The typical paper chromatograms of amino acids present in tomato juice are about the same as those of the amino acids present in soy sauce *(9)*. On acid hydrolysis, two additional amino acids were found, arginine and phenylalanine. Glutamine and asparagine were not present in the chromatograms of the hydrolyzate because they were hydrolyzed in acid solution to the respective amino acids. A total of 10 amino acids was found. Among these are 5 essential amino acids — arginine, leucine, phenylalanine, threonine, and valine. Serine may be considered as a semi-indispensable amino acid which is also present in tomato juice.

Ion-exchange chromatography. The free amino acids of tomato juice were determined quantitatively by ion-exchange chromatography, and the results are shown in Table 2. Attention was directed to those samples which showed higher increases in amino nitrogen due to the fertilizer application, which would give a better illustration of the quantitative changes of amino acids.

For a complete picture of the nitrogen distribution, the combined and free amino acids of one sample of tomato juice were determined (Table 3). The total amino acids were determined in the acid hydrolyzate. The quantity of the combined amino acids was calculated by subtracting the free from the total amino acids, assuming there was no destruction of the amino acids during acid hydrolysis.

The data shown in Table 2 indicate that application of fertilizers brought about an increase in some amino acids but no practical change in others. The

TABLE 2

Effect of field fertilizer treatment on the free amino acids of canned tomato juice

Treatment	Amino acids mg/100 ml. juice								
	Alanine	Asparagine Glutamine [1]	Aspartic acid	Glutamic acid	Glycine	Leucine	Serine	Threonine	Valine
I. 1956 season									
1. Control (no fertilizer)	13.90	23.20	27.50	172.00	18.25	5.95	16.40	6.60	15.00
2. NH_4NO_3 120 lbs. N/acre	39.70	62.25	50.60	213.00	11.50	6.10	14.10	34.60	6.50
4. NH_4NO_3, TSP K_2SO_4, 120 lbs. N, P_2O_5, K2O/acre	40.30	62.50	49.50	214.80	14.60	11.90	12.80	11.40	9.70
5. NH_3, 60 lbs. N/acre	38.10	37.00	22.70	176.00	19.00	8.20	15.65	8.25	26.50
6. NH_3, 120 lbs. N/acre	51.40	60.60	63.60	201.70	15.80	4.15	15.90	18.00	21.50
II. 1955 season									
1. Control (no fertilizer)	7.15	16.50	36.20	150.00	20.50	22.50	5.70	2.80	24.50
2. NH_4NO_3, 130 lbs. N/acre	26.60	22.80	60.30	173.00	6.95	11.20	8.45	7.75	11.00
3. NH_3, 130 lbs. N/acre	72.00	15.50	90.50	243.00	13.90	13.00	6.60	6.20	34.00

[1] Asparagine and glutamine appear together in the chromatograms.

TABLE 3
Distribution of amino acids in canned tomato juice
(Control Sample, 1955 season)

Amino acid	mg. of amino acid per 100 ml. juice		
	Free form	Combined form [1]	Total
Alanine	7.15	72.85	80.00
Asparagine Glutamine	16.50	— [2]	16.50
Aspartic acid	36.20	122.00	158.00
Glutamic acid	150.00	198.00	348.00
Glycine	20.50	14.30	34.80
Leucine	22.50	24.00	46.50
Phenylalanine	0	22.00	22.00
Serine	5.70	31.30	37.00
Threonine	2.80	25.00	27.80
Valine	24.50	18.00	42.50

[1] The combined amino acids (protein and peptide form) were calculated by subtracting the free form from the total amino acid (acid hydrolyzate).
[2] The two amides, on acid hydrolysis, give the respective amino acids and they are determined as such.

quantity of glutamic acid, aspartic acid, glutamine, asparagine, alanine, and valine increased to a certain degree. This increase is more definite in the sample treated with a high amount of anhydrous ammonia (No. 6).

Two typical chromatograms showing the relative amounts of the amino acids in the control and in the fertilized sample are shown in Figures 1 and 2. The same trend was found in a series of samples of the 1955 pack, although there was some variation in the absolute quantity of each amino acid.

Glutamic acid is the major amino acid in tomato juice. Aspartic acid, asparagine, glutamine, alanine, and valine are also present in considerable amounts.

DISCUSSION

It is not known if tomatoes produced under conditions favoring high yields differ in chemical composition from those grown under conditions of low yields. The results obtained in this investigation seem to indicate that there was no significant difference in soluble solids content among the samples given different fertilizer treatment. However, total nitrogen content of the fertilized tomatoes was, in every instance, higher than that of the unfertilized product. This indicates that when there is an abundance of the nutrient, which results in higher tonnage per acre, the tomato plant can take up more nitrogen into the fruit.

Amino acids in tomatoes have been investigated by the microbiological assay method by Lyman and Kuiken *(5)*. Because of the recent progress made by Moore and Stein *(7, 8)* in the field of ion-exchange chromatography, it is interesting to apply their method to tomato products. The results found here seem to agree with the results reported by Lyman and Kuiken *(5)* on some of the amino acids in tomatoes: arginine (.023%), histidine (.013%), iso-leucine (.020%), leucine (.028%), lysine (.028%), methionine (.006%), phenylalanine (.019%), threonine (.020%), tryptophane (.007%), and valine (.023%). They did not report glutamic, aspartic acid, and glycine because they were interested only in essential amino acids in foods. However, in the

present investigation the essential amino acids histidine, isoleucine, lysine, and methionine were not found. Alanine, aspartic acid, glutamic acid, glycine and serine were found by the ion-exchange method as used in this investigation, but were not investigated by Lyman and Kuiken. Results presented here indicate that glutamic acid is the major amino acid in tomato juice. Application of fertilizers brought about an increase in some amino acids but no practical change in others. It seems likely that the amino acid composition of tomato fruit differs to some extent with the mineral nutrition in the soil.

In Table 3 it is shown that most of the amino acids were present in the combined form rather than the free form. This condition was more evident in the case of glutamic and aspartic acids. Glutamic acid appeared to be the most abundant of all the free amino acids. It may serve as a donor of an amino group in transamination reactions. In addition, mono sodium glutamate, a known strong flavor constituent, could contribute a great deal to the flavor of tomato juice.

It should be noted that the paper chromatography and ion-exchange methods identify only the ninhydrin positive compounds. A different analytical procedure is needed for the determination of certain other nitrogenous compounds, such as pyrrolidonecarboxylic acid *(12)*. Proline and hydroxyproline, which give yellow colors with ninhydrin, were not estimated with this reagent. Possingham *(11)* identified proline in tomato shoots by spraying the paper chromatogram with a saturated solution of isatin in ethanol and then heating at 65° C. for 30 minutes. Under this condition only proline and hydroxyproline give blue colors. This test was not used in the present investigation. In the present work arginine was not detected in the free state, but its presence was evident in the acid hydrolyzate. Its absence in the free form might be caused by its chemical reaction with reducing sugars during storage through the browning reaction. It is known that arginine and other basic amino acids are far more reactive with reducing sugars in the Maillard type of browning reaction *(2)*. Storage of the canned product, especially when the storage temperature is high, accelerates this reaction.

Tryptophane has been reported by some workers to be present in tomato plants and fruits but not in processed tomato products. This amino acid was not detected in the present investigation, nor was Possingham *(11)* able to find tryptophane in tomato shoots. Its absence in canned tomato juice may be explained by the destructive effect of heat in acid medium on this amino acid during processing. Alkaline hydrolysis of the tomato juice proteins also failed to show a detectable amount of tryptophane.

SUMMARY

The effect of inorganic fertilizer application on yield and amino acid composition of tomatoes has been investigated. Yield increased from 22.3 tons per acre in the control sample to a maximum of 33.7 tons in a fertilized sample, the increase varying with the type and quantity of fertilizer applied. Total nitrogen content in the tomato fruit increases with increased amounts of nitrogen applied in the field. Two-dimensional paper chromatography indicated the presence of the following free amino acids in canned tomato juice: alanine, asparagine, aspartic acid, glutamic acid, glutamine, glynine, leucine,

serine, threonine, and valine. Acid hydrolysis of the tomato juice revealed the presence of two additional amino acids, arginine and phenylalanine.

Free and combined amino acids in canned tomato juice were determined quantitatively by Dowex 50 x 4 cation-exchange column chromatography. Glutamic acid is the major amino acid in tomato juice. Aspartic acid, asparagine, glutamine, alanine, and valine are also present in considerable amount. Application of fertilizers brought about an increase in some amino acids but no practical change in others. The quantity of glutamic acid, aspartic acid, glutamine, asparagine, alanine, and valine increased to a certain degree. This increase is more definite in samples treated with a high amount of anhydrous ammonia.

Acknowledgment

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DETERMINATION OF SORBIC ACID AND ITS DISAPPEARANCE FROM PICKLE BRINES

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(Manuscript received March 28, 1958)

During the 1957 season commercial-scale trials were set up to test any effect sorbic acid might have, by reason of its well-known yeast-inhibiting action, on the CO_2 tension of cucumber pickle brines. Among the several properties which were followed was the persistence of sorbic acid during the fermentations. In this connection a study of sorbic acid spectra and distillation resulted in modifications of published analytical methods *(8, 9, 13)* which should permit greater convenience and reliability of sorbic acid analyses.

EXPERIMENTAL

Purification of sorbic acid and determination of absorption coefficients. Ten grams of Eastman practical grade sorbic acid were recrystallized twice from 15 to 20% ethyl alcohol in water by heating and cooling. The product was recrystallized 3 times more by suspending in boiling water and adding just enough ethyl alcohol to dissolve the suspension, followed by cooling to room temperature. Absorbance measurements were made on the 0X, 2X, 4X, 5X crystallized material dried under oil-pump vacuum at room temperature by weighing about 0.5 g. on an analytical balance, dissolving in ethyl alcohol to 25 ml. and diluting with glass-distilled water. A single, calibrated 5 ml. Normax pipette was used for all dilutions. Appropriate volumes of 1.01*N* HCl or NaOH were added to the final dilutions to make the solutions 0.0101*N* in acid or base. Spectra were scanned in 1 cm. cells in the Cary Model 14 recording spectrophotometer from 200 to 400 mμ against 0.0101*N* HCl or NaOH as reference. Molar extinction coefficients for the spectra in these two media are plotted in Figure 1, and absorption coefficients at the maxima (263 mμ in acid, 254 mμ in alkali) are listed for several stages of crystallization with some replication in Table 1. Purity as judged by spectrophotometric change was not increased significantly beyond the second crystallization. The 4X crystallized material had a melting point of 133.5 – 134.7° C. and a neutral equivalent of 112.3, 112.7, 112.5 (theor. = 112.16).

Stability of sorbic acid in 0.01*N* HCl and 0.01*N* NaOH. Solutions of 4X crystallized sorbic acid at 4.17 μg./ml. in 0.01 *N* HCl and 0.01 *N* NaOH were stored at room temperature in the light for 22 days without significant loss. Absorption losses at the maxima were as follows in 0.01 *N* HCl: 5 days, 0.2%; 7 days, 0.4%; 22 days, 0.3%; and for 0.01 *N* NaOH at 22 days a gain of 0.5% was measured.

Steam distillation of sorbic acid. The rate of steam distillation of 1.25 mg. of sorbic acid was determined for 2 different distilland volumes: first, 100 ml. of 0.1*N* H_2SO_4 + 100 g. $MgSO_4 \cdot 7H_2O$ and second, 200 ml. 0.1*N* H_2SO_4 + 200 g. $MgSO_4 \cdot 7H_2O$, by collecting the distillate in successive increments as indicated in Table 2.

Table 3 shows the distillation recovery for different distilland volumes, all carried out in commercial macro-Kjeldahl distillation units. The 50-ml. scale is clearly too small for this type of equipment which has a large ratio of inner surface to liquid volume. The 100-ml. scale, however, should be sufficient for routine analysis and was used in the sorbic acid determinations in pickle brine shown in Table 4. Distillations were carried out to incipient solidificaton of the distilland.

The rate of loss of sorbic acid by evaporation of its aqueous solutions at room temperature is very low compared to that at the boiling temperature of the salt solution used in the analysis. A solution of 417 μg. of sorbic acid per ml. in water lost 0.7% when 6.6% of its volume was evaporated and lost only 4.7% of its sorbic acid when 40% evaporated at room temperature.

[a] Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

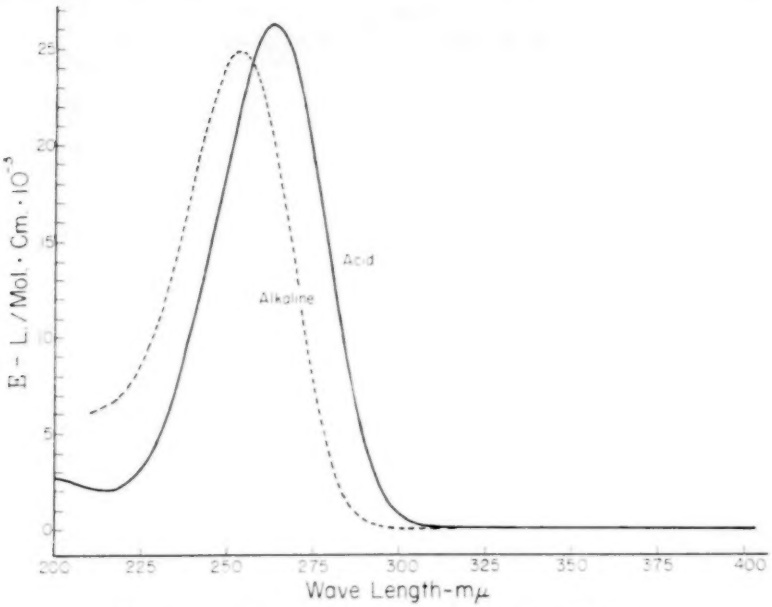

Figure 1. Spectra of sorbic acid in aqueous acid and alkali.

TABLE 1
Absorption coefficients of sorbic acid

No. of crystallizations	Absorption — liters/g.-cm. ($E^{0.1\%/1\,cm.}$)	
	0.01N HCl (263 mμ)	0.01N NaOH (254 mμ)
0	228.3	
	228.5	
2	233.2	222.0
4	234.5	221.6
	234.1	
	233.4	219.7
5	233.1	
	233.2	220.0

Assay procedure. To 3 ml. of cucumber brine (containing about 500 μg. of sorbic acid) in a macro-Kjeldahl flask are added 100 g. of $MgSO_4 \cdot 7H_2O$ and 100 ml. of 0.1*N* H_2SO_4. The mixture is distilled on a commercial Kjeldahl distillation rack with the usual self-draining spray trap until about 95 ml. of distillate have been collected in a 100-ml. volumetric flask or until the contents of the distillation flask start to solidify. The solidification point is preceded by a short period of foaming which is easy to recognize. Pyrolysis should be avoided because of the danger of the resulting decomposition products reaching the collected distillate.

TABLE 2
Rate of steam distillation of sorbic acid

Distilland volume	Successive fractions		Recovery (%)		Rate
			Single fraction	Cumulative	
	(No.)	*(%)*			*(% of total/ml.)*
200 ml.	1st	25.2	59.6	59.6	1.22
	2nd	23.6	31.5	91.1	0.7
	3rd	25.6	7.1	98.2	0.14
	4th	25.6	1.7	100.	0.035
100 ml.	1st	29.2	62.6	62.6	2.2
	2nd	34.2	33.2	95.8	1.0
	3rd	36.5	4.3	100.	0.1

TABLE 3
Distillation recovery of sorbic acid

Taken	Recovered	Distilland vol.
(mg.)	*(%)*	*(ml.)*
0.20	86	50
0.20	88	50
1.00	82	50
1.02	96.5	100
1.02	96.7	100
1.25 [1]	98.8	100
1.25	98.0	100
1.25	98.0	90
1.25	98.1	90
1.25	101.	200
1.25	98.4	200

[1] 1N H_2SO_4.

The distillate is acidified with 1.00 ml. of 1.00*N* HCl and made to the 100 ml. mark with distilled water. The absorbance of the acidified distillate is determined against 0.0100*N* HCl at 263 mμ. After subtracting the absorbance of a comparable blank sample without sorbic acid, the net absorbance at 263 mμ is converted to micrograms of sorbic acid, using the absorption coefficient 234 liter/gram-cm.

In our experience the blank of pickle brine plus reagents has corresponded to about 4 to 5 μg. of sorbic acid per ml. of brine and has been so constant that little risk of significant error would have incurred in running only occasional blank determinations. All spectrophotometric measurements in this work were made by scanning spectra from 200 to 400 mμ in a Cary Model 14 recording spectrophotometer.

Since commercial macro-Kjeldahl distillation equipment contains rubber connections, it is important to clean out the stills well by distilling water through them so as to wash out any UV absorbers released by the rubber. For analysis of very high precision the use of all glass apparatus with a lower ratio of inner surface to liquid volume and without the presence of rubber would be preferable. However, in view of the general precision level of absorbance measurements, the use of commercial Kjeldahl equipment is justified by its convenience for routine analyses.

From the data on Table 2 it appears that a slight gain in recovery at the expense of increased distilling time might be obtained by doubling the amounts of reagents (200 g. $MgSO_4 \cdot 7H_2O$ and 200 ml. 0.1*N* H_2SO_4). The U.S.P. grade of magnesium sulfate heptahydrate appeared to be as satisfactory as the A.C.S. grade in this application.

Fermentations and sampling. Commercial production tanks were made available through the kind cooperation of Nalley's, Inc., at their Tacoma, Washington, plant. The 6 pairs of tanks cited in Table 4 varied in raw material and other factors that need not be given here, but each pair was matched as closely as feasible under production conditions in

TABLE 4
Persistence of added sorbic acid in cucumber fermentations

Experimental pair	Age days	Sorbic acid (micrograms/ml.)				Peak yeast counts (log No. per ml.)	
		Center Sampling		Trough Sampling			
		Control [1]	Sorbic	Control [1]	Sorbic	Control	Sorbic
L5,6	7	4	103	4	98	5.7	3.5
	14	5	50	4	47		
	28	10	39	4	42		
D1,3	7	4	163	5	178	5.3	3.5
	14	7	118	7	115		
	28	6	20	4	20		
J9,10	7	6	109	7	81	5.4	5.0[2]
	14	4	28	4	3		
	28	4	0	4	0		
I,II	7	11	142	8	149	5.0	4.6 [2]
	14	4	79	4	74		
	28			6	40		
III,IV	7	6	149	4	157	6.0	3.7
	14	6	152	4	144		
	28			4	108		
92,93	7			6	236		
	14			6	163		
	28			4	95		

[1] Analyses of control fermentations without added sorbic acid have been included in this table to show their variation. These blank values have been taken into account in the values reported for experimental tanks with added sorbic acid.

[2] In sorbic tank J9 yeasts appeared (log No. = 4.6) between the 14th and 17th days, and were maintained thereafter at moderate numbers. In sorbic tank I the moderate count (log No. = 4.6) was observed on the 13th day; all other samplings gave low counts (next highest log No. = 3.8).

the period August 10 to September 10, 1957. All were of 2600-gallon or larger capacity, all were brined with 12% salt solutions which equilibrated to 4.4% solids (22° salometer) within about 2 days and were maintained thus for 4 weeks. Ambient temperatures of brines were in the range 17 to 23° C.

A solution of sodium sorbate was added to one tank of each pair immediately after the tanks were headed and mixed by circulation of the brine. The calculated initial concentration was 0.020% sorbic acid (200 mg. per liter volume of the tank to the top of the brine).

Brines were sampled as described by Etchells and Jones *(7)* from a vertical trough on the side of each tank, and from the center of the tank as well until a close correlation had been demonstrated. Sulfuric acid was added (to 1%, yielding approximately pH 1) to the samples before mailing to inhibit microbial activity. Up to 1 week elapsed before assay, at room temperature in the dark.

Peak yeast counts are shown in Table 4, but details of microbiological work will not be given here. Seven to 9 plate counts were made throughout a 25- to 30-day period after closing the tanks.

RESULTS AND DISCUSSION

Sorbic acid has been widely proposed as a food preservative because of its mold and yeast inhibiting action and its low toxicity. Its determination in low concentration is made relatively easy by steam volatility and its very high light absorption in the ultra-violet due to conjugated unsaturation. Published analytical methods for submilligram amounts are based on these properties.

There is considerable variation in the reported values *(6, 9, 11, 12)* for both the wavelength and extinction of the sorbic acid absorption maximum. While some of this variation is due to solvent differences and purity of the sample, a considerable amount appears to be due to lack of control of the two different spectra, one for the acid and another for the salts of sorbic acid (that is, sorbate ion). In view of the lack of agreement in the literature, the spectra of commercial all-trans *(6)* sorbic acid was measured from 200 to 400 mμ in 0.01*N* HCl and 0.01*N* NaOH and the acid was then recrystallized repeatedly to constant spectrophotometric behavior. The purification raised the specific absorbance about 2%. The acid and alkaline (salt) spectra of 4X crystallized sorbic acid are shown in Figure 1, where it is evident that the acid and alkali maxima differ by 9 mμ and their extinctions by about 6%.

Lack of control of acidity in the solutions whose absorbance is being measured can cause errors of two kinds in a test based in a single wave length: first, error due to the different absorbances at the maxima in acid and base, and, second, that due to the wave length shift of the spectrum with acidity. The first error would be at most 6%. The second kind of error could, however, be much higher. If, for example, the acid specific absorption coefficient and wave length were applied to a solution of the sorbic acid salt, an error of about 20% would occur. The method of Luckmann and Melnick *(8, 9)* avoids errors of this second type by recommending that, rather than a single absorbance reading, a series of readings be taken to locate the maximum and then that an arbitrary absorption coefficient be applied. The effort of locating the absorption maximum for each determination may be avoided readily by making the spectrophotometric readings in a solution of acid or alkali so that a known form of the sorbic chromophore is examined. To be sure, the shape of the absorption curve may be influenced by high blanks which should be investigated where present.

Luckman and Melnick call attention to the dependence of steam distillation yield on acidity of the distilland and recommend that highly buffered flood slurries, the pH of which is above 6, be acidified to pH 4.5. At pH 4.5 nearly half is still in the ionized state, a condition which would tend to slow the rate of steam distillation. The gross errors which can arise from uncontrolled acidity of the distilland can be avoided by routine addition of a strong non-volatile acid such as H_2SO_4 to the slurry being distilled. This practice eliminates the need of pH examination of individual sample slurries and, furthermore, should contribute to constancy of control samples, some of whose UV-absorbing constituents may also have a pH dependent steam volatility and spectra.

As shown in Table 2, spectrophotometric examination of successive increments of distillate from an acidified distilland indicates the early portions of the distillate to be rich in sorbic acid relative to the later portions. Thus a second distillation *(9)* after replenishing the water in the distilland is not necessary. This permits a simplification of the apparatus and manipulations for the steam distillation step, making possible the use of commercial macro-Kjeldahl distillation equipment commonly found in food laboratories. In the terminal stages of the distillation collection of distillate should be interrupted before pyrolysis occurs.

The data of Table 2 indicate the distillation recovery to be about 98% when the distilland charge is 100 ml. of 0.01*N* H_2SO_4 and 100 g. of $MgSO_4 \cdot 7H_2O$. A slight gain in recovery might be achieved by doubling the amount of reagents in the distilland and the volume of distillate collected. Sorbic acid is very stable in pure solution at room temperature in the light in both 0.01*N* HCl and 0.01*N* NaOH, no significant loss being observed in three weeks.

Scanning of spectra of untreated cucumber brines diluted in 0.01*N* HCl after 21 days fermentation showed them to have a peak in the same region as that of sorbic acid with absorbance equivalent to about 75 micrograms of sorbic acid per ml. of brine. Thus, elimination of the steam distillation treatment would not be desirable in the analyses of the usual low levels of sorbic acid in cucumber brines.

Sorbic acid determinations on the brines were made at the suggestion of J. L. Etchells, who had observed losses. The data of Table 4 show substantial losses of an initial 0.02% sorbic acid, complete in one tank and in excess of 50% in three others. Recently, Costilow *et al.* *(4)* have reported similar losses.

The slowness of disappearance of sorbic acid from dilute solutions by evaporation or through the influence of light, together with the variable disappearance from brines, suggests that the disappearance in fermenting brines may arise by microbial action. Sorbic acid is metabolized by various microorganisms *(10, 14, 15)*, including some yeasts. However, lactic bacteria such as occur in cucumber brines have not been observed to degrade sorbic acid *(5, 14)*. Costilow has emphasized the possibility of chemical degradation of sorbic acid in cucumber fermentations *(3)*.

Various investigations are under way to determine the effectiveness of sorbic acid in repressing yeast growth in cucumber brines *(1, 2)*. Such control was effective at the initial low level of 0.02% sorbic acid in the tanks investigated by us, except for the J9, 10 pair wherein the sorbic acid had disappeared by the end of two weeks. The counts of Table 4 show that if yeasts are implicated in the disappearance of sorbic acid, very low numbers suffice.

SUMMARY

The absorption spectra of sorbic acid have been measured in aqueous acid and alkali on material crystallized to constant spectrophotometric properties. The analytical method of Melnick and Luckmann has been modified by (a) steam distillation of sorbic acid from an acid medium, (b) absorbance determination on a known form of the sorbic chromophore, and (c) use of commercial macro Kjeldahl distillation equipment.

Large and variable losses were found in commercial cucumber brines treated initially with 0.02% sorbic acid.

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CYSTEINE DERIVATIVES IN MATURE ONION BULBS[a]

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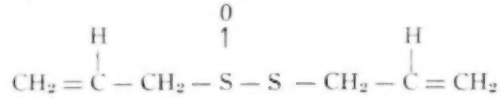
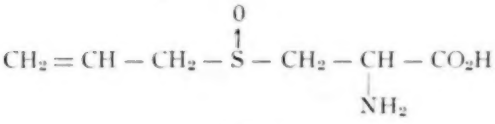
Steam distillation of macerated bulbs of the common onion yields a volatile oil with a pungent and lasting odor *(6)*. On the basis of previous research *(7, 8, 13)* on chemical composition, onion oil appeared to contain allyl propyl disulfide as the chief constituent, thiopropionaldehyde or thioallylaldehyde as the lachrymatory principle, hydrogen sulfide, propionaldehyde, and n-propyl thiol. A recent investigation concerning the chemical composition of onion volatiles by Niegisch and Stahl *(12)* has involved the application of mass spectrometric and infrared spectroscopic analyses to various fractions of onion emanations obtained by low temperature distillation and gas partition chromatography. Of the ten components reported, five were sulfur-containing compounds with n-propylthiol present in abundance and hydrogen sulfide, sulfur dioxide, dipropyl disulfide and possibly β-hydroxy propanethiol occurring in lesser amounts. No allyl propyl disulfide was reported present.

Our interest in this area was in regard to the nature of non-volatile precursors of the various volatile sulfur compounds reported from onions. Stoll and Seebeck *(14, 15)* in a similar study of volatile production in garlic, isolated a compound which served as the precursor to allicin, the anti-bacterial principle of garlic oil *(2, 3)*. This precursor was shown to be a sulfoxide derivative of cysteine, S-allyl-cysteine sulfoxide, and given the name alliine.



Allicin
[Diallyl thiosulfinate]



Alliine
[S-allyl-cysteine sulfoxide]

Cleavage of alliine by alliinase, an enzyme preparation from garlic, resulted in the formation of allicin, pyruvic acid and ammonia. Pyruvic acid production under these conditions is of considerable interest since Morgan *(9)* and Bennett *(1)* have reported appreciable amounts of pyruvic acid in onion juice and only small amounts in intact onion tissue. Vilkki *(16)* examined onions in an attempt to identify a pyruvic acid precursor and reported the presence

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of a sulfur containing amino acid, which when added to fresh onion juice, both increased the pyruvic acid content and fortified the onion odor. This compound was reported to be identical with the alliine of garlic.

This report is concerned with an examination of mature onion bulb tissue for the presence of alliine and other non-volatile sulfur compounds which might be related to the production of volatile, organic sulfur compounds.

EXPERIMENTAL

Chromatography of known sulfur amino acids. Preliminary paper chromatographic examination of cation fractions of onion extracts showed the presence of several areas representing sulfur-containing amino acids making it necessary to obtain chromatographic data with known compounds for comparison purposes. Methionine, glutathione, cysteine, cystine, homocysteine, cysteic acid, methionine sulfone, and methionine sulfoxide were obtained from commercial sources. Alliine, S-allyl-cysteine, S-propyl-cysteine sulfoxide, and S-propyl-cysteine were synthesized according to the methods of Stoll and Seebeck *(14)*. Samples of each amino acid were spotted on Whatman No. 1 paper and chromatographed in a descending manner at room temperature. Three solvent systems were used: 2,4-lutidine-water (65 : 35 v/v); phenol saturated with water; and n-butanol-acetic acid-water (4 : 1 : 1 v/v). After drying the papers in a fume hood, chromatographic positions of the various sulfur compounds were determined by spraying with ninhydrin and iodoplatinate *(17)*. A comparison of the chromatographic behavior of the sulfur amino acids tested is given in Table I which represents averages of at least two chromatograms from each solvent system.

TABLE I

A comparison of the chromatographic behavior of known sulfur amino acids

Compound	R value [1]		
	Solvent I [2]	Solvent II [3]	Solvent III [4]
Methionine	0.55	0.75	0.56
Glutathione	0.03	0.44	0.06
Cysteine	(streak)	0.16	0.07
Cystine	(streak)	0.18	0.07
Homocysteine	0.14	0.45	0.08
Cysteic acid	0.29	0.13	0.07
Methionine sulfoxide	0.18	0.78	0.25
Methionine sulfone	0.36	0.81	0.28
Alliine (S-allyl-cystine sulfoxide)	0.28	0.77	0.17
Desoxo-alliine (S-allyl-cysteine)	0.59	0.83	0.43
Dihydro-alliine (S-propyl-cysteine sulfoxide)	0.68	0.79	0.23
S-propyl-cysteine	0.78	0.85	0.51

[1] Whatman No. 1 Chromatographic paper, desending development, room temperature.
[2] 2,4-lutidine:water (65:35, v/v).
[3] Phenol saturated with water.
[4] n-butanol:acetic acid:water (4:1:1, v/v).

Preparation of onion and garlic bulb extracts. The general procedure followed was that of slicing mature, yellow onion bulbs into an equal weight of boiling water. After boiling for 2-3 minutes, the onion slices and water were transferred to a Waring blender and macerated for one minute. The macerate was then filtered through 4 layers of cheese cloth and 2 volumes of absolute ethyl alcohol were added with stirring to the resulting filtrate. The alcohol insoluble material was removed by centrifugation and the supernatant evaporated to dryness under reduced pressure at room temperature. The residue was redissolved in distilled water so that 1 ml. of the resulting solution represented the extract from 1 g. of onion bulb or garlic bulb tissue on a fresh weight basis.

Twenty milliliters of this extract was passed through a bed of cation exchange resin, 3 mm. by 160 mm. (Amberlite IR-120 [H^+]), adjusted to pass approximately 0.5 ml. per minute. The column was washed with water until the effluent showed a negative anthrone

test for carbohydrates. The cations were eluted from the column with 200-300 ml. of 5% ammonium hydroxide and the resulting solution of the "cation fraction" was evaporated to dryness in a rotary-type vacuum concentrator. The residue was dissolved in 1 ml. of water and served as the stock solution for paper chromatographic analysis.

Chromatographic comparison of onion and garlic bulb extracts. Aliquots of the "cation fractions" of garlic and onion extracts were spotted on Whatman No. 1 papers (18¼ x 22½ inches) along with samples of known sulfur-containing amino acids. The chromatograms were developed in a descending manner with n-butanol-acetic-acid-water (4:1:1 v/v) at room temperature. Figure 1 shows a composite tracing of a chromato-

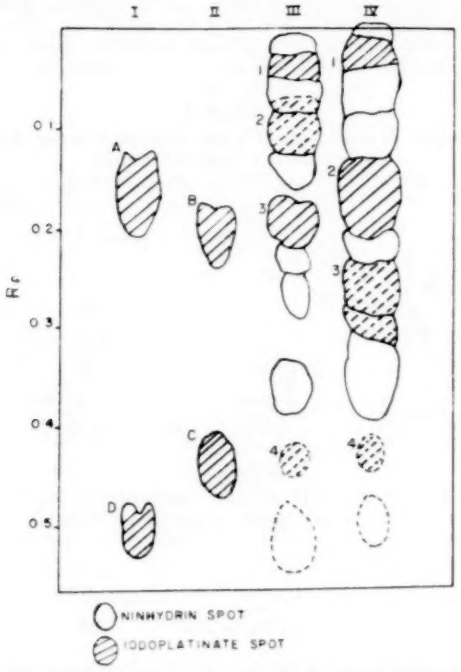


Figure 1. Composite chromatogram comparing ninhydrin and iodoplatinate reactive areas of cation fractions of mature garlic and onion bulb extracts with known cysteine derivatives. I—Alline (A) and S-propyl-cysteine (D); II—Dihydro-alliine (B) and desoxo-alliine (C); III—Onion cation fraction; IV—Garlic cation fraction. Broken lines indicate faint areas, Whatman 1. paper, n-butanol-acetic acid-water (4:1:1 v/v) solvent.

gram comparing the ninhydrin and iodoplatinate reactive areas on the chromatograms of garlic and onion "cation fractions". The chromatographic positions of synthetic alliine, S-allyl-cysteine, S-propyl-cysteine and S-propyl-cysteine sulfoxide are included for comparison.

Chromatographic identification of S-propyl-cysteine sulfoxide and S-allyl-cysteine. To obtain larger quantities of the onion compounds, aliquots of the cation fraction were applied as two-inch streaks at the starting line of chromatograms prepared from Whatman No. 4 or S & S No. 598 paper. The known cysteine derivatives, S-propyl-cysteine sulfoxide and S-allyl-cysteine, were applied on the same papers as marker spots between the streaks of onion cations. The bottom edge of the papers were serrated and the chromatograms developed in a descending manner for 16 hours at 25° C. with the n-butanol:acetic acid: water (4:1:1 v/v) solvent. After drying, the papers were cut lengthwise into strips

to separate the onion compounds from the synthetic compounds. The positions of the two known compounds were located on the paper strips by spraying with ninhydrin or iodoplatinate. The corresponding areas on the unsprayed strips containing the onion cations were cut out and extracted with distilled water. The extracts were combined, evaporated to dryness and finally redissolved in a few drops of distilled water. These two solutions served as a source material for the suspected S-propyl-cysteine sulfoxide and S-allyl-cysteine of mature onion bulbs.

Chromatographic identification of these eluted compounds in a number of solvent systems was carried out by ascending development on 9 inch squares of washed, Whatman No. 1 paper. The washing procedure consisted of successive treatment with water, dilute hydrochloric acid, the butanol-acetic acid solvent and finally water. Unknown onion compounds III-3 and III-4 (Figure 1) were chromatographed along with synthetic samples of S-allyl cysteine and S-propyl-cysteine sulfoxide in several solvent systems. The comparative R_f values obtained for the known and unknown compounds are shown in Tables 2 and 3.

TABLE 2

Comparison of R_f values of onion compound 4 and synthetic desoxo-alliine (S-allyl-cysteine)

Solvent	R values [1]	
	Onion compound "4"	Desoxo-alliine
n-Butanol:acetic acid:H_2O (4:1:1, v/v)	0.42	0.42
2,4-Lutidine:H_2O (65:35, v/v) [2]	0.51	0.50
Phenol sat'd. with H_2O [2]	0.76	0.76
Propanol:H_2O (89:20, v/v)	0.43	0.43
Pyridine:H_2O (65:35, v/v)	0.72	0.74
n-Butanol:ethanol:2N. HCl (4:1:2 v/v)	0.68	0.67

[1] Averages of at least two separate determinations with both ninhydrin and iodoplatinate spray reagents. Whatman 1 paper, ascending development.
[2] Only the ninhydrin reagent was used. Iodoplatinate caused the entire paper to turn blue.

TABLE 3

Comparison of R_f values of onion compound 3 and synthetic dihydro-alliine (S-propyl-cysteine sulfoxide)

Solvent	R values [1]	
	Onion compound "3"	Dihydro-alliine
n-Butanol:acetic acid:H_2O (4:1:1, v/v)	0.25	0.25
2,4-Lutidine:H_2O (65:35, v/v) [2]	0.46	0.46
Phenol sat'd. with H_2O [2]	0.79	0.79
n-Propanol:H_2O (80:20, v/v)	0.29	0.29
Pyridine:H_2O (65:35 v/v)	0.66	0.63
n-Butanol:ethanol:H_2O (4:1:5, v/v)	0.83	0.83
n-Butanol:ethanol:0.5 N. NH_4OH (7:1:2, v/v)	0.22	0.21

[1] Averages of 4 determinations for the known and at least two for the natural compound. Ninhydrin and iodoplatinate reagents used to detect compounds on the papers.
[2] Only the ninhydrin reagent used.

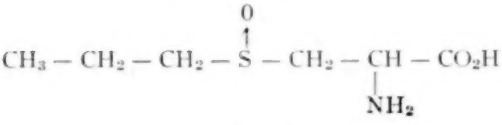
RESULTS AND DISCUSSION

In comparing the chromatographic behavior of a number of known sulfur containing amino acids (Table 1) the spot positions were detected by spraying the dried chromatograms with either the ninhydrin or iodoplatinate spray reagents. Ninhydrin treated papers showed discrete blue spots for all amino acids tested except for cysteine, alliine, and S-propyl-cystine sulfoxide which gave gray-blue spots. With iodoplatinate as the detecting spray reagent, all the sulfur-containing amino acids tested except cysteic acid and

methionine sulfone, appeared as white spots against a pink background. It is to be noted that although some cysteine derivates are not well separated by the phenol or butanol-acetic acid-water solvent they can be clearly separated by the use of the 2,4-lutidine-water solvent.

An examination of the chromatogram tracing (Fig. 1) comparing the ninhydrin and iodoplatinate reactive areas of "cation fractions" of extracts of onion and garlic bulbs indicates the possible presence of dihydro-alliine and desoxo-alliine in the onion fraction.

Desoxo-alliine
[S-allyl-cysteine]



Dihydro-alliine
[S-propyl-cysteine sulfoxide]

The principal garlic sulfur compound, IV-2, was shown to be identical with synthetic alliine on the basis of chromatographic behavior when eluted from the papers and rechromatographed with alliine in several solvent systems. This is in agreement with the findings of Stoll and Seebeck *(14, 15)*. No other garlic compounds were investigated, although the presence of desoxo-alliine in garlic is suggested.

Compound III-1 in onion and IV-1 in garlic (Fig. 1) reacted with both the ninhydrin and iodoplatinate spray reagents. Although this compound was not studied in detail, it gave a reddish color with ninhydrin characteristic of asparagine, and like asparagine, it also reacted with the iodoplatinate spray reagent. When asparagine was chromatographed on the same paper along with the onion cation fraction, it attained the same spot position as III-1.

The onion sulfur compounds III-3 and III-4, corresponding in chromatographic positions to synthetic dihydro- and desoxo-alliine respectively, were eluted from unsprayed chromatograms and rechromatographed in several different solvent systems. The comparative Rf values of the known and unknown compounds serve as chromatographic evidence for the identification of these onion compounds (Table 2, 3). Preliminary work employing only the phenol-water and butanol-acetic acid-water solvents strongly suggested that onion compound III-3 was alliine. The relatively poor chromatographic separation of alliine and dihydro-alliine obtained using these solvents may be seen in Table 1. The 2,4-lutidine-water solvent gave good resolution of these two compounds and indicated the absence of alliine in cation fractions of the onion bulb extracts being studied. The presence of alliine in garlic bulb extracts, on the other hand, was easily demonstrated by employing the same extraction, ion-exchange and chromatographic procedures used with the onion bulbs.

Although not yet investigated, the possibility that dihydro-alliine may be a precursor to the n-propyl mercaptan and dipropyl disulfide reported in onion volatiles by Niegisch and Stahl *(12)* is intriguing. An analagous cysteine derivative, L-S-methyl-cysteine sulfoxide, has been reported in cabbage by Dateo and co-workers *(5)* and shown to be the main precursor of dimethyl disulfide, the main constituent responsible for the odor of cooked cabbage. L-S-methyl-cysteine sulfoxide has also been isolated from turnip roots by Morris and Thompson *(10, 11)*. The possible role of desoxo-alliine and dihydro-alliine as precursors to volatile compounds in onion odors and to pyruvic acid in injured bulb tissue needs further study.

SUMMARY

Chromatographic evidence is presented indicating the presence of desoxo-alliine (S-allyl-cysteine) and dihydro-alliine (S-propyl-cysteine sulfoxide) in cation fractions of mature yellow onion bulbs. No alliine (S-allyl-cysteine sulfoxide) or S-propyl-cysteine was detected by the methods employed.

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CHANGES IN PECTIC SUBSTANCES OF FOUR VARIETIES OF PICKLING CUCUMBERS DURING FERMENTATION AND SOFTENING[a]

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It has been observed during studies on softening in salt-stock pickles *(13)* that there was a lack of correlation between the pectolytic enzyme activities of salt-stock brines and softness (as measured by the U.S.D.A. pressure tester) among different varieties of pickling cucumbers. Some varieties remained firmer than others in spite of high pectolytic enzyme activity in the brines.

The differences observed among varieties and the correlation of softening of salt-stock with the occurrence of pectolytic enzymes *(3)* in the fermentation brines instigated a study of the pectic substances of four varieties of pickling cucumbers at various stages of fermentation and softness.

EXPERIMENTAL

Preparation of samples. Cucumbers of the Ohio MR-17, Ohio MR-25, Wisconsin SR-6 and Wisconsin SMR12-12 varieties were grown on similar plots of soil at the University of Massachusetts[c]. Samples of these cucumbers were harvested and salted the same day. For the cucumbers intended to be classified as "firm" salt stock, cucumbers were salted in 25-gallon barrels using salting procedures previously described *(13)*. For the "soft" salt stock, 10½ pounds of cucumbers of each variety were put into two and a half gallon glass jars. Each jar was then filled with brine from a previous fermentation that had indicated extremely high pectolytic enzyme activity as determined by the viscosity method of Bell, Etchells, and Jones *(4)*.

Periodic examinations for pectolytic enzyme activity were made on the fermentations. Results are summarized in Table 1.

Pressure test examinations were made with a U. S. Department of Agriculture fruit pressure tester[d] on fresh unsalted fruit of the 4 varieties and on the salt stock. These

TABLE 1

Relative pectolytic enzyme activity in brines of firm and soft salt-stock for a two month period.

Variety	Firm salt stock	Soft salt stock
Ohio MR-17	0.20[1]	6.25
Ohio MR-25	2.96	7.28
Wisconsin SR-6	2.00	6.25
Wisconsin SMR12-12	4.31	7.14

[1] Expressed as areas (square inches) obtained from curve plotting per cent loss of viscosity vs. days of fermentation.

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[c] Courtesy of Professor C. L. Thomson, Department of Horticulture, University of Massachusetts, Amherst, Massachusetts.

[d] D. Ballauf Manufacturing Company, Inc., Washington, D. C.

TABLE 2

Pressure test results on four varieties of cucumbers at various stages of softness

Variety	Fresh, unsalted cucumbers		Relatively firm salt stock		Soft salt stock	
MR-17	20.6 [1]	S.D.	18.5	S.D.	14.5	S.D.
MR-25	19.9 S.D.[2]		16.7		12.0	
SR-6	18.8		14.7	S.D.	11.8	N.S.
SMR12-12	18.2		14.5	N.S.[3]	11.9	

[1] Expressed as average number of pounds required by pressure tester to penetrate fruit.
Ranges: 16 and higher — Firm
14 through 15.9 — Slightly Soft
below 14 — Soft
[2] S.D. means significant difference at five per cent level of confidence.
[3] N.S. means no significant difference at five per cent level of confidence.

results are shown in Table 2. From 30 to 45 cucumbers were used for each pressure test evaluation.

Tissue samples were prepared for pectic substance characterization from the fresh fruit, firm salt stock, and soft salt stock of each variety. Number two size (3¾ in. to 4¾ in. long) cucumbers were used in an attempt to obtain approximately the same maturity in all samples.

The following method of tissue preparation was used. Cucumbers were sliced with a hand-operated vegetable slicer into slices approximately one-eighth inch in thickness. To extract sugars and inactive enzymes, 600 g. of these slices were added to 2 l. of boiling 95% ethanol slowly so that the temperature of the mixture did not fall below 70° C. Salt stock cucumbers were not freshened prior to the alcohol extraction. The mixture of ethanol and cucumbers was heated for 15-20 minutes. Alcohol soluble materials were removed by filtering through gauze diaper cloth, and the tissue was allowed to stand for two hours in fresh 95% ethanol.

Alcohol was removed by filtering again through gauze diaper cloth. The sugar-free tissue was then dried at 65.5° C for 1½ hours on plastic screens in a tunnel dehydrator equipped with a blower. Tissues were stored under refrigeration in tightly sealed glass jars until needed for later extraction and characterization of the pectic substances.

Total pectic substances. Total pectic substances (as anhydrouronic acid) on a wet basis were determined on the fresh fruit and on the firm and soft salt stock by the method of McCready and McComb *(15)*. Results are shown in Table 3.

TABLE 3

Per cent total pectic substances (as anhydrouronic acid) in pickling cucumbers at various stages of fermentation and softness

Wet basis

Description of tissue	Variety			
	MR-17	MR-25	SR-6	SMR12-12
Fresh cucumber	0.265	0.241	0.222	0.219
"Firm" salt stock	0.223	0.209	0.217	0.199
"Soft" salt stock	0.250	0.262	0.292	0.256
Per cent change Fresh to "soft"	−5.7%	+8.7%	+31.5%	+16.9%

Fractionation of pectic substances. Pectic substances in the dried sugar-free tissues of the fresh fruit and of the firm and soft salt stock of the 4 varieties were removed by separate, successive extractions with distilled water (for pectin), 0.5% Versene at pH 6.0 (for pectinic acid) and 0.5NHCl at 85 ± 5° C. (for protopectin).

Six grams of dried, sugar-free tissue, ground to 30-mesh in a Wiley mill, were stirred with 600 ml. of distilled water for four hours. All extractions were made in sets of four (one of each variety) using a multiple stirring apparatus to insure uniform agitation. Trials revealed that the speed of agitation affected the amount of material extracted. At the end of the extraction time, the water-soluble extract was separated from the tissue by filtration with suction, using Whatman Number 41-h filter paper. The volume of each resulting extract was measured to enable a correction for the water remaining in the tissue.

The residue from the water extraction was suspended in 600 ml. of 0.5% Versene solution and the pH adjusted to 6.0 with concentrated HCl. This mixture was agitated for 4 hours, the extract filtered as for the water extraction, and the extraction procedure repeated with 600 ml. 0.5% Versene solution at pH 6.0. The volumes of the combined filtrates of the Versene solution extractions were measured for volume correction purposes.

Residue from the previous extraction was next suspended in 600 ml. of 0.5N HCl. The extraction mixture was heated to 85 ± 5° C. on a hot plate and extracted for 2 hours.

Six extractions of the water-soluble, Versene-soluble, and acid-soluble pectic substances were made from each of the varieties. Aliquots for anhydrouronic acid analysis were removed from each extract and made to volume in 100 ml. volumetric flasks, using 20 ml. 0.05N NaOH and water. Analyses were run on 2 ml. aliquots of the diluted extract by the carbazole procedure used previously for total pectic substances. Results, as per cent anhydrouronic acid in the tissues on an ash, water, and sugar-free basis, are summarized in Table 4. The total pectic substance value for each variety is the sum of the water-soluble, Versene-soluble, and acid-soluble fractions.

An attempt was made to precipitate the pectic substances from each fraction of each variety. It was observed that the amount of pectic substances that could be obtained from the water-soluble fraction would be insufficient for further characterization studies. Pectic substances of the acid-soluble fraction were precipitable in hot ethanol at pH 2.0, acetone, and benzene. However, the precipitate passed through the filter paper when attempts were made to separate it from the liquid portion of the mixture. This indicated that some degradation of the pectic substances had occurred and further characterization attempts would not give results indicative of conditions existing in the tissue.

Pectic substances from the Versene-soluble fraction were precipitated and separated. For each volume of extract two volumes of 95% ethanol were heated to boiling. Sufficient concentrated HCl was added to each extract prior to its addition to the boiling ethanol to bring the final pH to 2.0. The mixture was allowed to stand overnight at room temperature. The supernatant clear liquid was siphoned off and the remaining mixture filtered using suction. The precipitate was washed three times with 95% ethanol, twice with acetone, and allowed to dry at room temperature on a watch glass. It was then scraped off the watch glass, ground to 60-mesh in a Wiley mill and stored in tightly stoppered 25 ml. Erlenmeyer flasks.

Viscosities and methoxyl contents of Versene-soluble pectic substances. The relative viscosities of the Versene-soluble pectic substances of each variety were determined by the method of Owens *et al. (16)* at 25° C. using Ostwalde-Fenske Number 50 pipettes. Results are shown in Table 6.

The methoxyl (CH_3O—) contents were determined by the saponification method as outlined by Stier, Ball, and Maclinn *(19)*. See Table 7.

Calcium. Since calcium and pectic substances can unite in the form of calcium pectate, a gel, calcium determinations were made on the dried tissue of the fresh fruit, with and without the removal of sugar with ethanol, and on the dried tissue of freshened and unfreshened salt stock. Sodium determinations were made on the salt stock so that correction could be made for the sodium chloride content of the salt stock.

Analyses for calcium and sodium were made with the use of a flame photometer. Where sodium interfered with calcium analysis, calcium was determined by the calcium oxalate method as given by the A.O.A.C. *(1)*. See Table 8.

Pectolytic enzyme activity in fresh cucumbers. Bell *(2)* had reported the presence of pectolytic enzymes in ripe whole fruit of different cucumber varieties and intimated that the fruit may be a source of softening enzymes during fermentation. The method used for this study is a modification of that of Bell *(2)*.

Fruit for these examinations had been frozen and stored at 0° F. (− 18.7° C.) until the time of the test. Eighty grams of the partially thawed fruit were blended with 40 ml. of distilled water in a Waring blender until a loose slurry was obtained. Then 40 g. more

TABLE 4

Fractionation of pectic substances of pickling cucumbers at various stages of fermentation and softness

	Water soluble [1]				0.5% Versene-soluble [1]			
	MR-17	MR-25	SR-6	SMR12-12	MR-17	MR-25	SR-6	SMR12-12
	S.D.[2]							
Fresh cucumbers	2.05	2.11	2.37	2.38	5.49	4.89	4.10	4.34
	S.D.							
			S.D.	S.D.	S.D.			
Firm cucumbers	1.33	1.33	1.60	1.22	7.02	8.59	7.97	7.46
			S.D.				S.D.	
	S.D.				S.D.			
	S.D.	S.D.		S.D.		S.D.		
Soft cucumbers	0.77	0.88	1.02	0.93	9.57	9.65	11.28	10.38
	0.5N HCl soluble [1]				Total Pectic substances [1]			
	MR-17	MR-25	SR-6	SMR12-12	MR-17	MR-25	SR-6	SMR12-12
Fresh cucumbers	7.36	7.06	7.67	7.84	12.56	11.84	12.29	12.60
		S.D.[2]						
			S.D.					
Firm cucumbers	4.92	6.10	7.70	6.47	13.25	16.13	16.03	15.51
Soft cucumbers	2.56	2.31	2.70	2.67	13.00	12.89	15.04	14.05

[1] Expressed as per cent of the tissue on an ash, sugar, and water-free basis.
[2] S.D. means significant difference at 5% level of confidence.

TABLE 5

Per cent change in amounts of pectic substances in fractions extracted from pickling cucumbers at various stages of softness

Description of change	Water soluble fraction				0.5% Versene soluble fraction			
	MR-17	MR-25	SR-6	SMR12-12	MR-17	MR-25	SR-6	SMR12-12
From fresh cucumber to "firm" salt stock	−35.3	−35.2	−34.6	−48.1	+27.9	+76.5	+93.4	+72.0
From fresh cucumber to "soft" salt stock	−62.8	−58.1	−57.2	−61.1	+74.5	+97.5	+175.0	+139.0

S.D.[1] S.D. S.D. N.S.[2] S.D. S.D.

Description of change	0.5N-HCl soluble fraction			
	MR-17	MR-25	SR-6	SMR12-12
From fresh cucumber to "firm" salt stock	−33.1	−13.6	+0.4	−17.5
From fresh cucumber to "soft" salt stock	−65.2	−32.7	−51.7	−66.0

[1] S.D. means significant difference at five per cent level of confidence as determined by analysis of variance with interaction present.
[2] N.S. means no significant difference.

TABLE 6

Relative viscosities of 0.10% solutions of pectic (anhydrouronic acid) substances extracted by 0.5% Versene at pH 6.0

	Description of source of pectin		
	Fresh cucumber	Firm salt stock	Soft salt stock
MR-17	1.13	1.13	1.17
MR-25	1.19	1.15	1.21
SR-6	1.12	1.17	1.20
SMR12-12	1.23	1.19	1.20

TABLE 7

Per cent methoxyl in pectic substances extracted by 0.5% Versene solution at pH 6.0

	Description of source of pectin		
	Fresh cucumber	Firm salt stock	Soft salt stock
MR-17	5.8	4.7	4.0
MR-25	6.9	4.1	4.8
SR-6	5.7	6.6	5.4
SMR12-12	4.8	6.7	5.7

TABLE 8

Mineral contents of dried cucumber tissue from the fresh fruit and from salt stock

Tissue Description	Variety	Calcium per cent of tissue sample	Calcium on NaCl-free basis per cent	Calcium as per cent of A.H.A.
Fresh, sugar left in	MR-17	0.329		5.96
	MR-25	0.273		7.38
	SR-6	0.346		7.32
	SMR12-12	0.301		6.05
Fresh, sugar-free	MR-17	0.576		4.59
	MR-25	0.521		4.40
	SR-6	0.473		3.85
	SMR12-12	0.553		4.39
Salt stock, unfreshened	MR-17	0.116	0.710	6.82
	MR-25	0.088	0.858	5.33
	SR-6	0.113	0.692	6.81
	SMR12-12	0.128	1.248	8.95
Salt stock, freshened	MR-17	0.166	0.620	
	MR-25	0.136	0.445	
	SR-6	0.131	0.629	
	SMR12-12	0.149	0.715	

of fruit plus 3.2 g. sodium chloride were added to slurry and the mixture stirred until uniform. The extract was removed from the insoluble particles by filtration.

The extract was dialyzed for 3 hours in running tap water and one hour in distilled water to remove cations that could form gels with pectins. A portion of the dialyzed extract was adjusted to pH 3.0 and held for 24 hours at 40° C. as recommended by Bell *(2)* to inactivate pectinesterase. Tests were run on both the dialyzed unheated and dialyzed heated extracts.

Two standard 1.2% sodium polypectate solutions were made, one buffered at pH 4.1 and the other at pH 5.0. One ml. of extract was mixed with 5 ml. of each standard poly-

pectate solution and incubated for 20 and 44 hours. The viscosity of the mixture in each pipet was read at the time of mixing and at the end of each incubation period. See Table 9.

TABLE 9

Pectolytic enzyme activity of fresh cucumbers expressed as per cent loss of viscosity of a standard sodium polypectate solution

Variety	Extract dialyzed 4 hrs.					Extract dialyzed and held at 40° C. for 24 hrs.	
	pH 4.1	pH 5.0				pH 4.1	pH 5.0
MR-17	23.8	46.4				31.5	3.9
	24.8	45.7	S.D.	S.D.	S.D.[1]	32.3	23.2
MR-25	34.4	62.2				32.5	+75.4
	43.5	62.7				20.1	+52.9
SR-6	15.5	57.1				+37.7	+13.5
	41.0	58.2				+5.1	+23.4
SMR12-12	47.8	62.8				36.9	Gel.
	48.5	53.0				24.5	+48.8
Boiled Control	+13.2	4.8				—	—
	+12.5	6.1				—	—

[1] S.D. means significant difference at 1% level of confidence.

RESULTS AND DISCUSSION

Differences in the suitability of varieties of cucumbers for pickling have been shown by Ware *et al (20)*, Jones and Etchells *(9)*, and Jones, Etchells, and Monroe *(10)*. Their evaluations were based on firmness tests with the U. S. Department of Agriculture fruit pressure tester with notations made on susceptibility of the varieties to bloater formation. Table 2 shows that the 4 varieties in the present study differed in firmness both as fresh fruit and after curing in brines with moderate or extremely high pectolytic enzyme activities. See Table 1 for relative pectolytic enzyme activities of the brines.

A U. S. Department of Agriculture fruit pressure tester fitted with a 5/16 in. head was used for the pressure tests. It is admitted that skin toughness and size of seed area of the cucumbers vary with individual cucumbers and interfere with the true evaluation of firmness. However, the fruit pressure tester used with a large number (30 to 50) of samples from each variety did give results that agreed in general with organoleptic evaluation and characterization of firm and soft cucumbers and salt stock. It was concluded the fruit pressure tester is the best instrument available at the present time for evaluating firmness. Precaution was taken to use a large number of samples for a representative evaluation.

Pressure test results, summarized in Table 2, when compared with pectolytic enzyme activities of the brines, indicated that the presence of pectolytic enzymes, as shown by the loss of viscosity method of Bell, Etchells, and Jones *(4)* can cause softening of salt stock pickles. This is evident upon comparison of the general over-all pressure test drops from the "firm" to the "soft" salt stock. This agrees with the findings of Bell, Etchells, and Jones *(3)*.

These results also indicated that the significant differences in firmness among the fresh unsalted fruits of the different varieties carry through during the brining and curing in brines of either moderate or high enzyme activity. That is, the Ohio MR-17 variety, which was the significantly firmer fresh cucumber, remains firmer during fermentation in brines of high pectolytic enzyme activity. The Ohio MR-17 variety is significantly firmer than the Wisconsin SR-6 and the Wisconsin SMR12-12 varieties in all the tests run. The Ohio MR-25 variety can best be classified as slightly better than the softer varieties. Varietal differences, therefore, must be taken into consideration in the evaluation of salt stock for firmness and in the interpretation of the loss of viscosity tests for pectolytic enzymes as an indication of softness.

To determine exactly what changes occur in the cucumbers during softening, and because softening had been correlated to pectin-degrading enzymes, a study was made of the pectic substances of the 4 varieties of cucumbers for the fresh unsalted fruit, "firm" salt stock, and "soft" salt stock. An attempt was made to correlate varietal differences in firmness with varietal differences in pectic substances or changes in pectic substances.

First, total pectic substances were determined on a wet basis for the fresh fruit and the salt stock. Table 3 indicates that Ohio MR-17 may have more total pectic substances (as anhydrouronic acid) per unit volume of cucumber fruit than Wisconsin SMR12-12. The total pectic substances among the treatments (fresh, "firm" salt stock, and "soft" salt stock) cannot be directly compared because of the additional salt that had been absorbed during the brining of the salt stock. However, the salt concentrations among the salt stock pickles in each type of brine were about equal and a comparison of the changes in total pectic substances from fresh to "soft" salt stock was made. The changes in terms of percentages are given in Table 3. The greater increase in total pectic substances in the Wisconsin SR-6 and Wisconsin SMR-12-12 varieties may indicate a greater breakdown of pectic substances in those varieties than in Ohio MR-17.

The method of McCready and McComb *(15)* was used for these analyses. This method depends on de-esterification and enzymatic degradation of the pectic substances for extraction, and there is the possibility that the complete removal of the pectic substances is not accomplished by this extraction. More of the absolute amount of pectic substances may have been broken down in the softer varities to a form extractable by the reagents used for the analysis.

Results of these studies, either as to differences in amounts of or changes in total pectic substances, were limited and no statistical analysis of the data was attempted. Data may indicate differences that would be statistically valid if more replicates were run.

More detailed studies of the pectic constituents of fresh fruit and salt stock were made on dried, sugar-free tissue samples. The pectic substances of each tissue sample were fractionated into water-soluble (pectin), 0.5% Versene-soluble (pectinic acid), and 0.5NHCl-soluble (protopectin) portions. Results as per cent anhydrouronic acid in each fraction are listed in Table 4.

The general development of softness, as measured by the U. S. Department of Agriculture fruit pressure tester, correlates well with over-all changes in pectic substances soluble in Versene and dilute hydrochloric acid. There is

an increase in the Versene-soluble fraction and a decrease in the acid-soluble fraction, indicating a greater breakdown of protopectin to pectinic acid in the "soft" salt stock. The magnitude of these changes can be more readily seen in Table 5. These results agree with those of Fabian and Johnson *(7)* who found an increase in soluble pectin in spoiled pickles while the total pectic substances did not change.

Studies were made on alcohol precipitates from the Versene-soluble fractions for the degree of methyl esterification [as per cent methoxyl (CH_3O-)] and the relative viscosities of the pectic substances. Results of these tests, summarized in Tables 6 and 7, do not indicate conclusively whether or not any hydrolysis of the pectic substances of the Versene-soluble fraction occurred during fermentation or softening. Viscosities of extracts from fresh cucumbers were not much greater than those of the solvent indicating little viscosity to begin with. Also there may have been some degradation during the extraction, precipitation, drying, and redissolving of the pectic substances. Although there may have been some hydrolysis of the pectic substances in the pectinic acid fraction, there also was an increase in that fraction during fermentation and softening. The effect of this additional material from the breakdown of protopectin on the viscosity is not known. The contribution of partially hydrolyzed pectic substances to viscosity measurements is not known.

Jensen and MacDonnell *(8)* have advanced the hypothesis that at least two adjacent free carboxyl groups are required before polygalacturonase can hydrolyze the glycosidic bond associated with them. The present results on the degree of esterification indicate that such a possibility exists where two adjacent free carboxyl groups could easily occur. The maximum theoretical amount of esterification is 16.3% methoxyl. Assuming 16.3% methoxyl content is 100% degree of esterification, the present results show that only 24.6 to 42.3 of the theoretical maximum degree of esterification is found in the pectinic acids. This would then leave from 57.7 to 75.4% free carboxyl groups in the pectin.

Results of the tests for methoxyl content and relative viscosities, therefore, allow for the possibility of the existence of a polygalacturonase-like enzyme as was tested for in the brines. The present studies indicate that this type of enzyme or enzymes accompanying it also are responsible for the conversion of protopectin to pectinic acid. Examination of Tables 1, 2, and 5 point out the following correlations. With an increase in activity of a polygalacturonase-like enzyme in the salt stock brines, there is an accompanying decrease in firmness and an increase in the conversion of protopectin to pectinic acid.

Several recent reviews of pectic substances and pectic enzymes*(11, 12, 17)* report findings that substantiate this possibility. For many years it was believed that protopectinase was distinct from the enzymes that hydrolyzed pectinic acids. There is now the tendency toward the view that protopectinase and pectin-polygalacturonase are identical or that both exist in a pectic enzyme complex. These views are in harmony with the present belief that protopectin is either a modification of pectinic acid or is composed of very large pectinic acid molecules.

Table 5 shows differences in the changes of pectic substances among the four varieties. A statistical analysis of the changes (analysis of variance

with interaction present) of the pectic substances soluble in 0.5% Versene indicated that there was less increase in the pectinic acid fraction of the Ohio MR-17 variety during fermentation and softening than there was in Wisconsin SR-6 and Wisconsin SMR12-12 varieties. There was a difference between Ohio MR-17 and Ohio MR-25 during fermentation but not during softening. These differences would indicate less conversion of the protopectin to pectinic acid in the firmer variety. It would appear that the pectic substances of Ohio MR-17 cucumbers are of such a nature that they are more resistant to change during fermentation and softening.

Studies have shown that the characteristics of the pectic substances differ among varieties of the same vegetable or fruit for materials other than cucumbers. Kertesz *(12)* reported summaries of studies made with apples, peaches, bananas, and pears. Luh, Leonard, and Dempsey *(14)* compared the pectic substances of 3 varieties of tomatoes and found differences. Differences in the above studies were noted among varieties during ripening or storage.

There is no indication of variation among changes in the acid-soluble fractions of the varieties. It was impossible to control several variables encountered in this extraction; namely, uniformity of heat and uniformity of stirring speed. The extracting agent, aided by the application of heat, proved to be a strong one; there is the possibility that it did not quantitatively extract pectic substances. Whereas the other extractions were fairly uniform and represented trends, erratic results were obtained with this fraction.

Calcium can combine with certain types of pectic substances, and by forming a calcium pectate gel, contribute to firmness *(12, 18, 11)*. For this reason calcium analyses were made on the dried tissues of fresh cucumbers with and without the extraction of sugars by 95% ethanol, and on dried tissues of the "firm" salt stock, unfreshened and partially freshened.

Table 8 shows the results of analyses of 4 varieties grown and brined during the summer of 1956. The tissues listed in Table 8 are from the varieties and fermentations used for characterization of changes in pectic substances during fermentation and softening. To obtain the calcium contents of the salt stock on a basis by which they could be compared to those of the fresh tissues, the amount of sodium in the salt was determined, calculated as sodium chloride, and the calcium then given on a sodium chloride-free basis. The over-all higher results for the salt stock were due to the removal of fermentable or soluble matter from those tissues during the salting and fermentation.

If calcium had an effect on firmness in these studies, a higher calcium content, either as per cent of the total tissue or as per cent of the anhydrouronic acid in those tissues, would be expected in the firmer variety. Contrary to such opinion, the calcium seems to be higher in the softer salt stock, both unfreshened and partially freshened, than it is in the firmer variety. No correlation between firmness as measured by the fruit pressure tester and calcium contents of the tissues can be made.

As a final study of the nature of pectic substances in pickling cucumbers, the relative pectolytic enzyme activity of extracts of the fresh cucumbers were determined by the viscosity method used for detecting similar enzymes in the salt stock brines (see Table 9). An adaptation of the method of Bell

(2) to the method of Bell, Etchells and Jones *(4)* was made and used. All extracts were of equal dilution. The results indicated that a loss of viscosity occurred in a standard sodium polypectate solution for all extracts at pH 4.1 and 5.0. The loss of viscosity for the firm Ohio MR-17 variety was less at both pH values than it was for the other three varieties, indicating less pectolytic enzyme activity for the firmer variety.

Kertesz *(12)* criticizes prolonged tests, such as that of Bell *(2)*, on several points. Solutions of pectinic acids show gradual irreversible loss in viscosity even in the absence of enzymes. For this purpose the results with boiled controls are listed in Table 9. Also, the effectiveness of toluene as a preservative has been questioned. Determinations for pectolytic enzymes were made in duplicate; it is doubtful if such close agreement as was obtained could have been obtained if microbial contamination of an unknown degree had been present. The danger of contamination was lessened by incubating and determining viscosities without the need for transfer from a flask to a separate viscometer. Ostwalde-Fenske modified viscosity pipettes were used for both purposes. The main reason for using the viscosity method was that it was the method used for determining similar enzymes in the salt stock brines.

Etchells, Bell, and Jones *(6)* have implicated molds carried by blossoms adhering to immature cucumbers as a potent source of pectolytic enzymes in salt stock brines. Demain and Phaff *(5)* in a recent review conclude that there are potentially a number of sources of the enzyme (or enzymes) responsible for pickle softening. The most likely sources appear to be from molds and the cucumber plant itself. The present studies confirmed the possibility that the cucumbers themselves may be important sources of pectolytic enzymes.

The Ohio MR-17 variety showed significantly less pectolytic enzyme activity than the 3 softer varieties. Although the enzymes present in the cucumbers may not by themselves cause complete softening, they may act as auxiliary enzymes to those from other sources and thus hasten softening of cucumber salt stock.

SUMMARY

These studies on the pectic substances of different varieties of cucumbers at various stages of softness have partially explained the differences in firmness among varieties, both fresh and fermented, have helped explain the lack of correlation sometimes obtained between pressure tests for firmness and evidence of appreciable, pectolytic enzyme activity in the fermentation brines, and have characterized the changes in pectic substances that occur during softening.

The conclusions drawn from these studies are:

1. Pectolytic enzymes cause softening of salt stock pickles. However, certain varieties were inherently firmer than others as fresh cucumbers and remained firmer during fermentation even in the presence of high pectolytic enzyme activity in the brines.

2. The general development of softness in cucumber salt stock was accompanied by a conversion of the acid-soluble pectic substances (protopectin) to Versene-soluble pectic substances (pectinic acid). It was not possible

to determine whether further degradation of the pectic substances had accompanied this conversion.

3. Of the four varieties of pickling cucumbers studied from the firmness point of view, the Ohio MR-17 variety was the best. The Wisconsin SR-6 and Wisconsin SMR12-12 varieties were the softest and ranked equally low. The Ohio MR-25 rated an evaluation slightly better than the Wisconsin varieties. Factors included in this evaluation were the pressure test results for fresh and fermented cucumbers, resistance of the protopectin to breakdown during softening, and relative pectolytic enzyme activities of extracts of the fresh cucumbers.

In the presence of approximately equal enzyme activities, the protopectin of the Ohio MR-17 variety resisted conversion to pectinic acid to a greater degree than that of the softer varieties.

4. There was no correlation between calcium contents of the tissues and firmness.

5. A water extract of the fresh cucumber of the Ohio MR-17 variety indicated significantly less pectolytic enzyme activity than a similar extract from the other three varieties.

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THE FICIN CONTENT OF THE LATEX FROM DIFFERENT VARIETIES OF *FICUS CARICA* AND A COMPARISON OF SEVERAL MICRO-METHODS OF PROTEIN DETERMINATION*

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Ficin, a papainase type of enzyme, is present in the latex of the fig tree. Robbins and Lamson *(5)* determined the ficin content of sixteen species of the genus *Ficus*. They used the digestion of *Ascaris* and the increase in amino nitrogen of gelatin as assay methods. Only in two of the species examined was the enzyme concentration high enough to digest *Ascaris* in a 2.2% solution of the latex. The latex of *Ficus carica* L., the edible fig, was found to contain more proteolytic activity than any of the other fifteen species tested. The latex from six South American trees had high proteolytic activity while the latex from four genera of the family *Moraceae* contained less than one-fifth the proteolytic activity of *Ficus carica* L.

There are a large number of varieties of *Ficus carica* grown in this country. Yet there has not been any extensive work reported on the variation of ficin content of the latex with the variety of fig. In order to determine if variety has any effect on the proteolytic activity of the latex the ficin content of some 25 varieties of *Ficus carica* were determined and the results are reported in this paper. An evaluation of several micro-techniques of protein determination are also compared.

EXPERIMENTAL METHODS

Collection of fig latex. With the exception of the Kadota, Black Mission, and Stanford Caprifig varieties marked "Davis" the latex was collected from trees in the U. S. Department of Agriculture experimental plot at Fresno, California. Green figs were separated from the tree in such a manner that the stem was left on the tree. The several drops of latex from the fig were allowed to drip into a test tube. The latex was frozen by the use of dry ice right after collection and then stored at −25° C. until the samples could be analyzed.

Casein-digestion activity. A known amount of the thawed latex (allowed to thaw at 4° C.) was diluted to the proper range of activity with cold distilled water and the activity was determined at 35.0° C. by the method described previously *(7)* with the exception of one change. Because of the instability of the diluted enzyme at pH 7.0 in the absence of substrate, the enzyme was added to the reaction mixture last. One unit of casein-digestion activity is defined as the amount of enzyme which will produce an optical density change of 0.001 in one minute when carried out under the conditions and by the method described *(7)*.

Milk-clotting activity. The milk-clotting activity was determined by the method of Balls and Hoover *(1)* with certain modifications. The substrate was prepared from Carnation Instant Nonfat Dry Milk solids. The latex was diluted to the proper activity range with distilled water and in the case of the activated sample the solution was made 0.006 M with respect to cysteine and versene. Two-tenths milliliters of diluted latex was added rapidly (1-2 seconds) to 1.0 ml. milk in a 15 x 150 mm. test tube which had previously been equilibrated at 35.0° C. for 5 minutes. A timer was started at the beginning of the

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addition of the sample. The tube was rotated slowly at a 30° angle around its long axis and the time noted for the formation of a grainy texture in the layer on the tube wall. Duplicates, when run by this method, agreed within 0.5 to 1%. One unit of milk-clotting activity is defined as that amount of enzyme which will clot one ml. of milk in 60 seconds.

Determination of organic matter. This was carried out by the method of Johnson *(3)*. Crystalline egg albumin (Nutritional Biochemicals Corporation) and crystalline sucrose (Fisher Scientific Company), analyzed for their moisture content and in the case of egg albumin its nitrogen content, were used for the preparation of a standard curve. It is interesting that both compounds gave identical straight line curves.

Determination of nitrogen content. The protein content of the fig latices were determined by three methods: the total nitrogen by the micro method of Johnson *(2)* multiplied by 6.25, the Lowry method *(4)* and light absorption at 260 and 280 mμ *(6)*. Crystalline bovine serum albumin and crystalline egg albumin, analyzed for their moisture and nitrogen content, were used for the preparation of standard curves in the Lowry method.

RESULTS AND DISCUSSION

Stability of the proteolytic activity of fig latex. Table 1 shows the effect of storage of 1-2 ml. samples of latex in 15 x 150 mm. test tubes at 23-25° C. and at — 25° C. on the stability of ficin. Very little activity was lost after 60 days of storage at — 25° C.; the enzyme was much more labile at 23-25° C. Freezing and thawing did not have any effect on the enzymatic activity as shown by a comparison of the activity initially before freezing and after freezing and thawing 17 days later (Table 1). The data show that the enzyme became inactivated at room temperature for two reasons; first, there was a reversible loss in activity because of the oxidation of the essential sulfhydryl group, and second, there was an irreversible loss in activity of the enzymes.

Casein-digesting and milk-clotting activity of fig latex. Table 2 shows the specific casein-digesting and milk-clotting activities of latex from 27 sources. The varieties Fraga, California Brown Turkey, Black Mission (1951), Adriatic, Martinique, Conadria, and Kadota (1950) were uncaprified while the remainder were caprified. Caprification versus non-caprification did not appear to have any effect on the proteolytic activity of the latex. However, a difference was found in the proteolytic activity of the latex from the Black Mission (1951) grown at Fresno which was not caprified and the Black Mission (Davis) grown at Davis which was caprified. The difference in location may be responsible for the difference found. Only about a two-fold difference was found in the proteolytic activity of the latices from all the sources. Activated commercial ficin was found to have an activity of 938 units of casein-digestion activity per mg. so the activity of most of the latices tested compare quite favorably with it. It should be noted that commercial ficin has had most of the lipid material removed from it by "fractionation" and has been treated differently from the latices in this study so that the results are not strictly comparable.

The ratio of casein-digesting activity to milk-clotting activity was found to be a constant with the exception of two latices. The ratios of the activities for the latices from the King and Stanford Caprifig were found to be 228 and 168, respectively. The variation from the average is greater than three times the standard deviation. It is interesting to note that the ratio of casein-digesting to milk-clotting activity for commercial ficin, which was obtained from a wild variety of fig in South America, is around 150.

TABLE 1

Retention of proteolytic activity of Stanford caprifig latex (Davis) measured by the casein digestion method

Storage at room temperature (23-25° C)					Storage at − 25° C.				
Days	Not activated		Activated		Days	Not activated		Activated	
	Units/mg. organic matter	Activity lost	Units/mg. organic matter	Activity lost		Units/mg. organic matter	Activity lost	Units/mg. organic matter	Activity lost
		(%)		(%)			(%)		(%)
0	470	—	570	—	0	470	—	570	—
2	463	1.5	565	0.9	17	468	0.4	571	− 0.2
6	408	13.2	546	4.2	30	466	0.9	565	0.9
17	143	69.6	411	27.9	60	462	1.7	566	0.7

TABLE 2

The casein-digesting and milk-clotting activity of fig latex

Variety	Milk-Clotting Enzyme units/mg. organic matter		Casein-Digestion Enzyme units/mg. organic matter		Ratio casein-digestion to milk-clotting activity (activated)
	Not activated	Activated	Not activated	Activated	
45-105 [1] (1950) [2] +++ [3]	2.77	3.83	945	1260	329
184-15 (1953) +	2.97	3.42	775	1110	325
Kassaba (1950) +	3.20	3.26	940	1080	331
Blanquette (1950) ++	2.70	3.19	841	981	308
Fraga (1950) ++	3.39	3.28	838	954	291
Calimyrna (1953) +++	2.82	2.98	701	906	303
Calif. Brown Turkey (1950) +++	1.90	2.38	732	900	378
Marabout (1950) ++++	2.49	2.68	706	895	334
Black Mission (1951) ++	2.18	2.86	732	892	312
194-21 (1956) ++	2.41	2.47	586	891	358
Adriatic (1950) +++	2.68	2.50	731	886	354
Martinique (1950) ++	2.20	2.89	563	844	292
202-6 (1956) ++	2.25	2.64	522	842	319
Snowden (1950) +	2.74	2.64	688	814	308
135-15 (1953) +++	1.32	2.23	300	805	361
Conadria (1953) ++	2.03	2.41	444	804	333
Pied de Boeuf (1951) ++++	1.40	2.83	275	800	282
135-4 (1953) ++	2.32	1.66	475	785	338 [4]
Kadota (1950) +++	1.63	2.51	521	779	310
King (1950) ++	2.14	3.36	689	766	228 [5]
Black Mission (Davis) +++	2.05	2.44	532	745	305
38-64 (1950) ++++	2.34	2.02	482	703	348
Kadota (Davis) +++	1.25	2.09	454	692	331
195-21 (1956) ++	1.04	2.14	187	672	314
156-28 (1955) +++	1.51	2.02	214	661	327
Bourabat (1950) +++	1.80	2.18	354	656	301
Stanford Caprifig (Davis) +++	2.29	3.40	470	570	168 [5]
Average					324
Standard deviation					± 23

[1] These are experimental varieties.
The seedlings and parents are listed for some of the experimental varieties. Par means parthenocarpic fruit.

194-21 Kadota X 71-53 (Par x Par violet capri)
195-21 Kadota X 70-95 (Par x Par green capri)
184-15 71-4 X 71-90 (Par x Par ♂71 Partridge Eye x 35-2, both Par)
135-4 Victory X Excelsior (Non Par x Non Par)
135-15 Victory X Excelsior
202-6 Mission X 76-11 (Par x Par; Purple x Violet)

[2] The number in parenthesis refer to the year the tree was planted or grafted onto a root stock.
[3] The + refers to the relative ease of obtaining the latex from the fig; for example ++++ means several drops were collected from one fig while + means only one or two drops could be obtained. This was a function of the thickness of the latex.
[4] The unactivated milk clotting value was used.
[5] Not included in the average.

Organic matter and protein content of fig latex. The average organic matter of the latices from the different sources was found to be 50.3 ± 7.5% (Table 3). It should be mentioned that the standard deviation, $S = \sqrt{\frac{\Sigma d^2}{n}}$, as used in this report does not reflect the precision of the methods used, but rather the variation among the samples. All values are the average of at least 3 determinations which agreed quite closely.

TABLE 3
The organic matter and protein content of fig latex

Variety	Organic Matter per ml fig latex (grams)	Grams protein/gram organic matter			
		Nitrogen content x 6.25 (a)	Lowry Method		Absorption at 280 mu (d)
			Standard cryst. egg albumin (b)	Standard bovine serum albumin (c)	
45-105	0.394	0.446	0.414	0.472	1.08
184-15	0.432	0.345	0.368	0.419	0.785
Kassaba	0.472	0.396	0.390	0.440	0.814
Blanquette	0.649	0.270	0.265	0.302	0.596
Fraga	0.573	0.302	0.309	0.352	0.674
Calimyrna	0.476	0.406	0.368	0.418	0.957
Calif. Brown Turkey	0.537	0.304	0.266	0.306	0.581
Marabout	0.582	0.275	0.277	0.314	0.609
Black Mission	0.514	0.288	0.305	0.350	0.678
194-21	0.402	0.358	0.334	0.383	0.779
Adriatic	0.481	0.291	0.295	0.339	0.569
Martinique	0.537	0.302	0.304	0.346	0.720
202-6	0.550	0.322	0.286	0.326	0.713
Snowden	0.500	0.328	0.318	0.364	0.838
135-15	0.420	0.293	0.279	0.322	0.738
Conadria	0.537	0.278	0.296	0.337	0.636
Pied de Boeuf	0.322	0.296	0.342	0.394	0.644
135-4	0.461	0.282	0.267	0.306	0.610
Kadota	0.510	0.292	0.279	0.320	0.520
King	0.588	0.264	0.242	0.277	0.602
Black Mission (Davis)	0.488	0.293	0.272	0.313	0.584
38-64	0.619	0.310	0.284	0.324	0.701
Kadota (Davis)	0.588	0.248	0.219	0.253	0.482
195-21	0.420	0.288	0.257	0.295	0.524
156-28	0.551	0.258	0.243	0.279	0.464
Bourabat	0.588	0.230	0.252	0.289	0.684
Stanford Caprifrig (Davis)	0.386	0.201	0.214	0.245	0.620
Average	0.503	0.302	0.294	0.336	0.676
Standard deviation	±0.075	±0.053	±0.048	±0.054	±0.136
Ratio			(b)/(a) = 0.974	(c)/(a) = 1.11	(d)/(a) = 2.24

There are a number of methods for determining the protein content of a solution, and this work offered an opportunity to compare some of them. The protein content was determined by the nitrogen content times 6.25, the Lowry method using both crystalline egg albumin and crystalline bovine serum albumin as standards, and by the comparison of the light absorption at 260 and 280 mμ.

The nitrogen content times 6.25 gave an average value of 30.2 ± 5.3% for the protein content of the latices. There are two disadvantages in the determination of protein by this method. (a) The method determines the total nitrogen of the samples and not just the protein nitrogen. This disadvantage can largely be overcome by precipitating with trichloroacetic acid and determining the nitrogen content of the precipitate. This was not done in this work because of the small amount of protein present. (b) The factor 6.25 is an average value and in actual cases the nitrogen contents of proteins range

from 14 to 19%. If the nitrogen content of the protein is known the determination of the protein content by this method can be greatly improved.

The Lowry method gave an average protein content of 28.4 ± 4.8% (crystalline egg albumin as standard) and 33.6 ± 5.4% (crystalline bovine serum albumin as standard). Thus the use of one standard gives a result lower than the nitrogen method and the other gives a result which is higher. The lower value is probably nearer the true value. There are two major disadvantages to the Lowry method. (a) The amount of color varies with different proteins as shown above for egg albumin and bovine serum albumin. In this respect it gives less constant values than the biuret method which were not used here because of the amount of protein required for this method. But it gives more constant values with different proteins than does the ultraviolet absorption at 280 mμ which will be described below. (b) The color is not strictly proportional to concentration. There are, however, certain advantages to the Lowry method. It is more convenient and as sensitive as the digestion and subsequent nesslerization used in the nitrogen method above. It is 10 to 20 times as sensitive as measurement of the ultraviolet absorption at 280μ, and it is much more specific and less liable to inaccuracy due to turbidity. It is 100 times as sensitive as the biuret reaction.

The ultraviolet absorption at 280 mμ gave an average protein content of 67.4 ± 13.6%. This value is twice that obtained by any of the other methods. The protein content was calculated according to an equation derived from the data of Warburg and Christian which is

$$\text{protein concentration (mg./ml.)} = 1.55\ D_{280} - 0.76\ D_{260} \qquad (1)$$

where D is the optical density of the solution.

Nucleic acids, usually present in tissue extracts, have a strong absorption at 280 mμ. However, nucleic acid absorbs much more strongly at 260 mμ than at 280 mμ, whereas the reverse is true for proteins. This can be used to eliminate, by calculation, the interference by nucleic acid. As shown by the data here, considerable error may be involved in the use of this method since different proteins and nucleic acids do not always have the same absorption. Other substances such as purine and pyrimidine nucleotides may also exhibit absorption in the region from 260 to 280 mμ. Turbidity may also be a problem. However, the estimation of protein can be carried out quickly and easily on a small amount of material and under conditions, i.e., in the presence of ammonium sulfate or other salts, which make other methods useless. The method is very useful for following the purification of a protein and where the true extinction coefficient is known exactly (as is true of many crystalline proteins) it offers a very rapid and accurate measure of the protein concentration.

The protein content of the fig latices have been treated as an average in order to compare the various methods used for its estimation. There are real differences in the protein content of the different latices. For example, the Stanford Caprifig organic matter is composed of 20.1% protein while the experimental variety 45-105 has 44.6% protein.

SUMMARY

The casein-digesting and milk-clotting activity of latices from 27 sources have been determined. There is about a two-fold variation in the casein-

digesting activities. The ratio of the casein-digesting and milk-clotting activities appears to be a constant for 25 of the latices tested.

The stability of the proteolytic activity of fig latex is discussed.

The organic matter and protein content of the latices were determined. An evaluation of the nitrogen method, the Lowry method and the absorption of 280 mμ for the determination of the protein content of a sample has been given.

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THE EFFECT OF VARIETY AND MATURITY ON THE PROTEOLYTIC ENZYME CONTENT OF FIGS*

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The effect of variety on the proteolytic enzyme content of the latex from the edible fig tree, *Ficus carica* L., has been discussed in a previous publication *(3)*. The ficin used in this country for the tenderization of meats, clarifying of beverages, etc., is imported from South America. In order to obtain the ficin, the wild fig tree is cut down, the latex which oozes out is collected, allowed to separate into the aqueous and lipid layers and the aqueous layer dried. This is commercial ficin. This method is not feasible in the United States because of the high costs of collecting the latex. However, there are several thousand tons of domestic figs and prunings which must be discarded each year and these may represent a cheap source of ficin.

Unfortunately for the fig industry, there has not been any information published on the ficin content of the fig. A knowledge of the amount of ficin present and the effect of variety and maturity on the ficin content of the fig are of paramount importance in any attempt to answer the question of the feasibility of obtaining ficin from the fig on a commercial scale. The purpose of this research is to show the effect of variety and maturity on the proteolytic enzyme content of the fig and to show that the fig does contain enough ficin to make it profitable to look for a way of extracting it from the fig.

EXPERIMENTAL METHODS

Collection of figs. The figs were obtained from the U. S. Department of Agriculture experimental plot in Fresno, California and from the Davis campus of the University of California. Quantitative recovery of the latex of the fig was assured by placing a small piece of tissue paper over the broken area immediately upon separation from the tree. The figs were separated as they were collected into 4 categories; very green, green, straw ripe, and ripe depending upon their size and appearance. Samples were stored at − 25° C. until they were analyzed.

Extraction of the proteolytic activity from the fig. It was found that the proteolytic activity could best be extracted from the figs by grinding them in a mortar with twice their weight of 0.50 N, pH 4.0, buffer containing equivalent amounts of borate, citrate and phosphate. The homogenate was then filtered through cheese cloth. When extracted by this method the proteolytic activity was stable up to 5 hours at room temperature and lost only 14.4% of its original activity on storage for 48 hours. When the pH of the extracting medium was around neutrality, the enzyme was rapidly inactivated. A large portion of this activity could be restored by addition of activators such as cysteine, but not all. The homogenation of the material in a Waring blender also produced some inactivation because of the air which was blended in. It was also found that a better extraction of the enzyme could be obtained with the buffer solution at room temperature than at 0° C. The effect of the pH of the extracting medium is shown in Table 1; the stability of the enzyme in the extracting medium is presented in Table 2. All values reported for the proteolytic activity represents an average value for duplicate determinations on at least two figs analyzed independently.

Determination of proteolytic activity. The enzyme content was determined by the

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TABLE 1

Effect of extracting medium on the proteolytic activity obtained from the Kadota (Davis) fig [1]

Extracting Medium	Initial ativity (Units) (Activated) [2]	Activity after 4 Hrs. at 4° C. (Units) (Activated)
0.16 N Each Borate, Citrate, Phosphate Buffer, pH 4.00	9.74	9.70
0.16 N Each Borate, Citrate, Phosphate Buffer, pH 5.00	9.75	8.61
0.16 N Each Borate, Citrate, Phosphate Buffer, pH 6.00	9.60	8.15
Distilled Water	0.99	——

[1] Extracted as described in the text but at 4° C. Activity expressed in casein-digesting units.
[2] Activated as used in this paper refers to the addition of 0.025 M cysteine and 0.025 M versene to the reaction medium for the purpose of activating the proteolytic enzymes.

TABLE 2

Stability of the proteolytic activity of California Brown Turkey fig extract at room temperature (23-25° C) [1]

Time	Activity (casein-digesting units per mg. fig)			
	Not activated	Activity lost	Activated	Activity lost
		%		%
Initial	10.1		12.5	
5 hours	10.2	− 1.00	12.5	0.00
1 day	6.75	33.2	11.5	8.00
2 days	5.52	45.4	10.7	14.4
4 days	4.48	55.6	7.89	36.8

[1] Extracted by the method described in the text with pH 4.00 buffer (0.16 M with respect to each borate, citrate and phosphate).

casein-digestion method described previously *(4)*, with the modification noted in a previous publication *(3)*. Values for activity in the tables are expressed in casein-digestion units. One casein-digestion unit is defined as that amount of enzyme which will produce an optical density change of 0.001 per minute when carried out by the method used here.

Determination of the organic matter. This was carried out on the filtrate by the method of Johnson *(2)* using crystalline egg albumin (Nutritional Biochemicals Corporation) and crystalline sucrose (Fischer Scientific Company) for the preparation of a standard curve. Values given for the organic matter represent the amount of material present in the filtrate after it had passed through the cheese cloth and not the total organic matter of the figs. In the tables it is expressed as per cent of the weight of the fig. This value, although not a true measure of the solids content of the fig, does give a value for the extractable solids as well as an indication of the maturity of the fig — as can be seen from the tables. It also provides a basis on which to express the nitrogen content of the fig. It is felt that this gives a fairly accurate measurement of the total nitrogen content of the fig. And finally, this value is representative of the solids content of the filtrate which was used for determining the enzymatic activity present. All values reported represent the average of at least 3 determinations.

Determination of the nitrogen content. The nitrogen content of the filtrate was determined by the method of Johnson *(1)* carried out in duplicate. It is expressed in terms of per cent of the organic matter found.

RESULTS AND DISCUSSION

Location of the proteolytic activity in the fig. In what part of the fig is the proteolytic activity located? If a green fig is examined it is found that

the milky latex is present only in the receptacle portion of the fig, not in the seed area. On the basis of this one might postulate that the proteolytic activity is located in the receptable portion only. In order to prove this, several figs were separated into the receptacle and the seed portions. Only the receptable portion was found to contain an appreciable amount of proteolytic activity as shown in Table 3.

TABLE 3

The location of the proteolytic activity in the fig (Kadota figs from Davis)

Fig No. and section	Weight	Units activity/mg. fig		Units activity/total weight x 10^{-3}		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Fig 1							
Half	8.30	2.20	3.93	18.3	32.6	7.48	3.21
Seeds	4.20	0.360	1.08	1.51	4.54	7.28	3.18
Receptacle Portion	3.75	5.52	8.84	20.7	33.1	4.43	5.15
Fig 2							
Half	10.75	1.87	4.25	20.1	45.7	4.85	4.90
Seeds	4.75	0.036	0.540	0.171	2.57	6.22	4.17
Receptacle Portion	5.50	4.14	7.32	22.7	40.2	7.28	3.48
Fig 3							
Half	10.20	1.57	3.35	16.0	34.2	5.38	3.75
Seeds	6.10	0.054	0.091	0.330	0.555	8.44	3.13
Receptacle Portion	5.50	3.42	6.64	18.8	36.5	4.75	4.57

Effect of variety and maturity on the proteolytic activity of figs. Table 4 shows the proteolytic activity of 22 varieties of figs at the very green and green stage of maturity. The numbers refer to experimental varieties of edible figs. An indication of the nature of these numbers is given in a previous publication *(3)*. It is seen that the varieties examined varied about 4-fold in their ficin content at the very green stage of maturity. Thus, more variation was found in the ficin content of the figs than in the latex *(3)*. A little calculation will show that, on a weight basis, the latex contained 30-50 times more activity than did the fig. In some varieties the proteolytic activity was greater in the green figs than in the very green figs while in others the reverse was found to be true. It would be hard to draw any conclusions as to the effect of maturity from this data. The extracted organic matter amounted to an average value of about 8.5% of the total weight of the fig at both stages of maturity while 3.46% at very green stage and 3.25% at the green stage is nitrogen. Therefore, some 22% of the organic matter is protein.

The varieties, Fraga, Martinique, and Conadria in Table 4, and Kadota, Adriatic, California Brown Turkey, and Black Mission in Tables 6, 7, 8, and 10 were not caprified while the remainder of the varieties were caprified. A comparison of the proteolytic activity of the caprified versus the uncaprified varieties shows that the uncaprified varieties tended to have a greater amount

TABLE 4

Effect of variety and maturity on the proteolytic activity of figs

Maturity		Very green					Green			
Variety	Average weight	Units activity/mg. fig		Organic matter	Nitrogen	Average weight	Units activity/mg. fig		Organic matter	Nitrogen
		Not activated	Activated				Not activated	Activated		
	(grams)			%	%	*(grams)*			%	%
Blanquette	8.82	9.81	14.0	10.4	3.27	12.08	12.0	15.2	10.6	2.83
Fraga	8.52	9.53	13.6	7.06	3.71	12.00	8.52	9.40	7.28	3.22
Marabout	10.40	8.62	13.5	7.48	4.13	21.28	7.52	10.1	11.3	1.96
Martinique	2.78	10.8	13.4	10.1	4.45	5.05	9.16	13.2	8.12	5.71
45-105	6.98	10.9	12.8	8.90	3.61	11.80	9.14	10.1	10.1	2.77
38-64	11.50	12.3	12.4	8.85	4.27	12.88	10.4	12.4	10.2	3.68
184-15	6.65	8.43	11.1	8.78	4.47	12.02	9.17	11.6	8.24	3.60
Conadria	8.18	9.57	11.0	8.65	3.77	10.02	7.60	12.0	7.06	4.13
202-6	6.40	8.75	10.7	7.02	4.08	15.15	8.36	10.5	6.96	3.85
194-21	4.08	6.57	9.24	7.24	3.43	17.08	7.34	8.81	8.17	4.26
Kassaba	10.28	7.17	9.00	9.70	3.13	17.10	3.42	5.24	8.89	2.86
Bourabat	9.68	6.63	8.90	——	——	16.28	6.83	9.62	8.28	3.55
156-28	8.12	7.16	7.98	9.02	2.20	18.60	8.14	9.60	9.06	2.76
Pied de Boeuf	5.80	4.95	7.93	7.42	2.62	13.50	4.24	6.98	8.01	2.30
195-21	3.45	6.13	7.58	7.39	2.52	11.60	4.74	7.56	8.59	2.68
Stanford Caprifig (Davis)	3.15	5.21	7.18	6.28	4.75	——	——	——	——	——
200-43	7.25	5.26	6.90	11.8	3.17	11.15	3.48	6.16	8.22	3.88
King	8.62	5.08	6.60	11.2	2.40	12.65	6.24	7.20	8.74	1.86
135-4	5.44	2.23	4.67	7.98	2.78	12.35	5.14	7.94	7.06	3.24
Snowden	13.70	3.02	4.38	——	——	17.68	2.33	3.62	9.07	2.56
135-15	6.29	1.87	2.96	6.66	3.03	14.48	5.68	7.27	6.17	3.08
64-105	——	——	——	——	——	10.22	8.98	11.4	9.06	3.43

Activated commercial ficin — 938 units per milligram.

TABLE 5

Effect of size and maturity on the proteolytic activity of Kadota figs [1]

Maturity	Weight	Units activity/mg. fig		Units activity/fig x 10 $^{-3}$		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Very green	3.00	5.04	7.10	15.1	21.3	6.75	4.73
Very green	4.85	5.50	7.26	26.7	35.2	6.96	4.22
Very green	7.20	5.15	7.19	37.1	51.8	6.54	4.27
Very green	11.50	5.19	7.10	59.6	81.6	5.06	4.60
Very green	14.45	6.83	8.64	98.6	125	5.90	4.61
Very green	15.10	4.72	6.70	71.3	101	5.38	3.96
Green	20.80	5.78	7.52	120	156	6.11	4.81
Green	21.90	3.75	5.80	82.2	127	4.64	4.52
Green	25.40	2.54	4.45	64.5	113	7.70	3.00
Green	32.50	1.39	2.86	45.2	93.0	6.85	3.70
Green	36.05	0.992	2.65	35.7	95.5	9.17	2.47
Straw ripe	53.40	0.018	0.973	0.962	52.0	14.7	1.81
Straw ripe	74.05	0.000	0.847	0.000	62.6	15.8	1.62
Ripe	22.80	0.000	0.775	0.000	17.7	22.7	1.63
Ripe	28.40	0.000	0.631	0.000	18.0	36.4	1.07

[1] Figs were obtained from the Davis Campus and were caprified.

TABLE 6

Effect of size and maturity on the proteolytic activity of Kadota figs [1]

Maturity	Weight	Units activity/mg. fig		Units activity/fig x 10 $^{-3}$		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Very green	3.75	8.12	10.5	30.4	39.4	9.70	2.99
Very green	4.60	10.2	12.5	46.9	57.5	7.80	3.86
Very green	5.30	10.7	14.8	56.7	78.4	7.48	4.94
Very green	5.75	11.8	16.6	67.9	95.5	8.32	3.80
Very green	6.00	9.75	12.3	58.5	73.8	8.33	3.62
Very green	6.90	9.69	11.8	66.9	81.5	10.9	2.32
Green	9.25	3.44	5.84	31.8	54.0	12.3	2.00
Green	11.20	3.23	5.66	36.2	63.4	9.90	2.08
Straw ripe	13.30	1.32	3.39	17.6	45.0	20.6	1.51
Straw ripe	11.80	0.306	1.28	3.62	15.1	24.0	1.15
Ripe	18.50	0.162	1.24	3.00	23.0	28.0	0.878
Ripe	11.15	0.000	0.000	0.000	0.000	33.2	1.01

[1] Figs were obtained from Fresno and were not caprified.

of activity per unit weight. However, the caprified figs, at the same level of maturity, were larger than the uncaprified figs and this difference disappears if the ficin content of the total fig is considered.

It may be rather difficult to realize the amount of proteolytic activity in a fig when the activity is expressed in casein-digesting units as it is in the tables. Perhaps the following will give a better idea of the ficin content of an average size (10-15 g.) green fig. An average size green fig of most of the varieties was found to contain activity equivalent to 100-150 mg. of commercial ficin. This is sufficient enzyme in one green fig to clot 1000-1500 ml. of milk in one minute.

TABLE 7
Effect of size and maturity on the proteolytic activity of Adriatic figs

Maturity	Weight	Units activity/mg. fig		Units activity/fig x 10 $^{-3}$		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Very green	4.00	10.3	12.0	41.2	48.0	9.17	2.88
Very green	6.00	10.3	12.5	61.8	75.0	9.06	3.52
Very green	7.35	9.23	11.5	67.9	84.5	9.17	2.93
Very green	7.55	9.50	11.7	71.7	88.4	8.44	3.62
Very green	7.65	9.32	10.3	71.4	78.8	8.65	2.70
Green	8.25	9.53	10.3	78.6	85.0	8.85	2.33
Green	8.95	9.69	12.2	86.7	109	9.38	2.62
Green	10.70	7.11	9.15	76.1	98.0	10.8	1.91
Green	10.80	7.50	9.03	81.0	97.6	10.4	2.22
Straw ripe	22.65	1.66	2.42	37.6	54.8	25.0	0.616
Straw ripe	24.60	1.41	3.17	34.7	78.0	29.6	0.764
Ripe	11.65	2.04	3.87	23.8	45.2	30.9	0.690
Ripe	18.00	0.360	0.666	6.48	12.0	32.6	0.715

TABLE 8
Effect of size and maturity on the proteolytic activity of California Brown Turkey figs

Maturity	Weight	Units activity/mg. fig		Units activity/fig x 10 $^{-3}$		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Very green	5.05	11.6	12.6	58.6	63.6	7.38	4.04
Very green	7.25	11.2	12.5	81.2	90.6	7.17	3.99
Very green	7.60	13.5	16.7	103	127	8.12	4.04
Very green	7.90	12.7	14.1	100	111	7.58	4.75
Very green	8.10	13.4	15.3	109	124	7.38	4.98
Green	10.55	12.1	14.6	128	154	6.95	4.49
Green	13.90	12.0	14.6	167	203	8.01	3.76
Green	14.10	6.21	7.76	87.6	110	7.80	2.64
Straw ripe	21.75	0.613	2.14	13.3	46.5	15.6	1.29
Straw ripe	23.25	0.144	1.37	3.35	31.9	15.6	1.27
Ripe	18.35	0.144	0.613	2.64	11.3	20.6	1.27
Ripe	18.70	0.144	0.450	2.69	8.42	26.1	0.950

Effect of size and maturity. Tables 5-10 show the effect of size and maturity on the proteolytic activity of Kadota, Adriatic, California Brown Turkey, Calimyrna and Black Mission figs. Of the varieties mentioned above, the Kadota (Davis) and the Calimyrna were caprified. The activity per unit weight of the Calimyrna, Kadota (Davis), Black Mission and Adriatic figs was found to remain more or less at a constant value up to the straw ripe stage while the activity per unit weight of the Kadota and California Brown Turkey figs was found to reach a maximum at a certain stage of maturity and then dropped off. The large green figs were found to contain the most activity per fig. Therefore, one would want to harvest the figs at this stage of maturity for production of the optimum amount of ficin.

TABLE 9

Effect of size and maturity on the proteolytic activity of Calimyrna figs

Maturity	Weight	Units activity/mg. fig		Units activity/fig x 10 $^{-3}$		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Very green	5.05	7.48	9.07	37.8	45.8	6.11	5.07
Very green	6.70	7.30	8.64	48.9	57.8	6.85	5.04
Very green	7.40	7.14	8.35	52.8	61.8	6.22	5.65
Very green	8.15	7.18	8.65	58.5	70.5	4.85	6.58
Very green	9.55	6.25	7.62	59.7	72.7	5.27	6.83
Green	12.70	6.13	8.50	77.9	108	7.16	4.73
Green	13.80	6.30	8.47	87.0	117	7.80	4.25
Green	14.65	6.79	8.52	99.5	125	4.85	6.75
Green	21.75	5.60	7.01	122	153	4.95	6.12
Straw ripe	43.35	0.162	0.433	7.02	18.8	11.6	1.23
Straw ripe	73.00	0.036	0.396	2.62	28.9	18.7	0.845
Ripe	52.80	0.144	0.540	7.60	28.5	31.3	0.978
Ripe	48.10	0.090	0.504	4.33	24.2	32.4	0.979

TABLE 10

Effect of size and maturity on the proteolytic activity of Black Mission figs [1]

Maturity	Weight	Units activity/mg. fig		Units activity/fig x 10 $^{-3}$		Organic matter	Nitrogen
		Not activated	Activated	Not activated	Activated		
	(grams)					%	%
Very green	5.05	10.4	12.0	52.5	60.6	6.75	4.16
Very green	6.20	8.89	10.3	55.1	63.9	7.58	3.92
Very green	7.50	10.9	12.6	81.7	94.5	8.21	3.18
Very green	7.95	9.60	10.7	76.4	85.1	6.85	4.45
Green	10.05	6.32	7.85	63.6	79.0	7.28	3.02
Green	12.00	8.03	9.90	96.5	119	7.70	2.82
Green	13.80	6.30	8.47	87.0	117	7.80	4.25
Green	14.25	5.90	7.25	84.1	103	7.07	2.59
Green	18.39	5.20	6.98	95.6	128	7.28	2.83
Straw ripe	19.30	1.84	2.96	35.5	57.2	14.7	1.18
Straw ripe	25.05	0.685	1.57	17.2	39.4	19.8	0.890
Ripe	13.40	0.577	1.84	7.73	24.7	34.5	0.990
Ripe	17.25	1.91	3.39	33.0	58.5	40.4	0.865

[1] Figs were obtained from Fresno and were not caprified.

As the fig reached the straw ripe stage, the proteolytic activity was found to decrease in all of the varieties studied. But it did not disappear completely even in the ripe fig; the remaining activity being some 10-20% of the original amount present. This is still sufficient enzyme in a fig to clot 140-160 ml. of milk in one minute.

The measurement of the organic solids gave an indication of the maturity of the fig as shown by the tables. The extractable solids were found to increase from a value of around 8% for the green fig to 30-40 per cent for the ripe fig depending upon the degree of dehydration. This simply represents an increase in the sugar content as the fig matured. The per cent nitrogen was found to decrease as the fig matured but when expressed on an absolute basis

it will be seen that it remained about constant throughout the growth and maturation of the fig.

Proteolytic activity of dried figs. The ripe figs for which data are given in the last section were obtained from the trees and had undergone only a slight amount of dehydration. Samples of sun-dried figs were obtained and analyzed for proteolytic activity. As shown by the data in Table 11, sun-

TABLE 11

Proteolytic activity of dried figs measured by the casein digestion method

Variety	Ave. Weight	Units activity/mg. fig		Units activity/fig x 10^{-3}		Organic matter	Nitrogen
		Not activated	Activated	Not Activated	Activated		
	(grams)					%	%
Adriatic	14.76	0.551	1.18	8.14	17.4	42.3	0.608
Conadria	10.05	0.802	1.35	8.06	13.6	45.0	0.860
Calimyrna	17.88	0.458	0.736	8.20	13.2	32.2	1.03
Kadota	9.82	0.515	0.947	5.05	9.30	34.2	1.09
Black Mission	8.98	0.628	1.34	5.65	12.0	42.2	0.805
184-15	13.5	0.918	1.45	12.4	19.6	37.8	1.56

dried figs still contained approximately 12% of the activity originally present in the figs at their optimum peak of activity. However, processed dried figs did not contain any activity.

Proteolytic enzymes do not exist completely in the active form in figs. In green figs the enzymes were found to be 75% active on the average. This value dropped to about 50% for straw-ripe figs and 20-30% for tree ripe figs. This indicates that the natural activators of the enzymes decreased as the fruit matured. About 50-60% of the enzymes were found to exist in the active form in the sun-dried fruit. The reason for this high value is not known; it is rather unexpected.

Although the fig contains only about 2% as much activity as the latex, there is a large amount of enzyme present. It appears that there is a good possibility of obtaining ficin from figs commercially. As mentioned, it does not appear feasible to obtain ficin from the latex in this country. If a method could be worked out for extracting the ficin from the fig, from the prunings of the tree and perhaps from fig shoots grown for this purpose, then the ficin produced by this method should be able to compete successfully with the other sources of ficin and with the suppliers of papain and bromelin. That such a method can be worked out has been shown in the case of the Hawaiian Pineapple Company and its production of bromelin.

SUMMARY

A four-fold variation was found in the ficin content of the varieties of figs studied. The proteolytic activity per unit weight of fig remained about constant up to the stage of straw-ripe. As the fig ripened the ficin content decreased to about 10-20% of the amount present at the optimum stage of maturity. This optimum stage of maturity is the large-green stage. Even sun-dried figs retained approximately 12% of their original amount of activ-

ity. The green fig contains sufficient ficin to make it profitable to look for a method of extracting the material.

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EFFECT OF TEMPERATURE AND BRUISING ON SUGAR AND ACID CONTENT IN FREE-STONE PEACHES

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It was determined earlier *(17)* that free-stone peaches become relatively sweeter and less tart within 24 hours after being bruised. Because there is an absolute decrease in the percentage of acid in the bruised fruit, respiratory changes appear to be involved. Consequently, environmental factors which can influence the rate of respiration might be expected to play a rôle in the sugar and acid metabolism of the wounded fruit tissues. Of such factors, that of temperature was chosen for further investigation.

Temperature relations during storage of normal, unbruised peaches have been studied by Bigelow and Gore *(4)*, Haller and Harding *(6)*, and Neubert, Schultz, Bedford and Carter *(12)*. These investigators have observed no consistent trends in the content of sugar and acid as affected by temperature. According to Haller and Harding *(6)*, the percentual amount of sugars will vary, depending on the relative rates of both transpiration and respiration at different temperatures. Likewise, the acid content is said to fluctuate considerably as a function of temperature. Similar types of irregular variations were recorded by Bigelow and Gore *(4)*, depending also upon the fruit variety. Although sugar content was indifferently affected, Neubert *et al. (12)* indicated that the acid content of stored peaches decreases with time. No specific thermal effects on acids were described for the range of temperatures used ("room temperature", 75°, 85°, 95° F.). Studies of bruising in cherries by Pollack *et al. (15)* have not been concerned with the respired substrate.

MATERIALS AND METHODS

Three separate collections of fruit were made from a single tree of a "seedling" variety of free-stone peach in late August, 1957. On each collection day, 40 peaches were segregated into groups of 8 for storing at 5 different temperatures. Half of each fruit was bruised thoroughly *(16)*, and the unbruised half was lighty marked before the fruit was placed in storage. The sample groups were held for 20 hours at the following temperatures: 34°, 70°, 95°, 115°, and 130° F. and frozen at − 15° F. (Blowers were operated continually in all storage rooms). The bruised halves in each group were separated from the unbruised halves along the fruit sutures while still frozen, and all were placed in polyethylene bags and held at − 15° F. for analysis.

Moisture, sugar, and acid contents were determined on a composite sample for each sample group. The analyses were made according to the schedule outlined earlier *(17)*, *i.e.*, free acid by titration to neutrality and sugars by the alkaline ferricyanide-ceric sulfate method.[a]

RESULTS AND DISCUSSION

Table 1 presents the analytical results of the experiment. Although care was taken to leave the peach skin intact during the bruising operation, the bruised halves tend to lose slightly more moisture than the corresponding un-

[a] The author is grateful for the help of R. E. Hughes, Jr. in performing these analyses.

TABLE 1

Sugar, acid, and moisture content of bruised and unbruised free-stone peaches

Storage Temperature	Treatment [1]	Content		
		Moisture	Sugar [2]	Acid [2, 3]
(F.°)		*(%)*	*(%)*	*(%)*
130	C	86.9	38.9	1.44
	B	86.8	40.8	1.45
115	C	87.4	43.3	1.89
	B	87.3	38.6	1.57
95	C	87.8	36.1	2.47
	B	87.6	32.7	1.84
70	C	88.6	41.4	2.91
	B	88.8	40.7	2.17
34	C	88.6	39.5	2.90
	B	88.4	34.7	2.90

[1] C = unbruised; B = bruised
[2] On a dry weight basis
[3] Computed as malic acid

bruised halves. More definitely, there is a progressive decrease in moisture content with increasing temperature. It is quite probable that the actual decrease, which is due to transpiration or evaporation, is much greater than that measured. Hence the metabolism of organic materials has not only provided additional moisture directly for the fruit but has also created an apparently higher moisture content indirectly by the disappearance of solid materials. (A significant volume decrease, together with the presence of fluid exudate, was observed in peaches held at the highest temperatures.)

If the peaches at 34° F. are regarded as being essentially unaltered after 20 hours of storage, the fluctuations of sugar content with temperature (and bruising) are not constant. In the absence of other overriding considerations, it is probably reasonable to assume that there is no principled difference in sugar content between bruised and unbruised peaches. The slightly lower amount of sugar in most bruised samples here is counterbalanced by the slightly higher amount found in the earlier study *(17)*. Since the proportionate amount of sugar does not vary greatly at different temperatures, it can also be assumed that the rate of sugar metabolism parallels the rate of water loss at the temperatures used.

In correspondence with these data, Miller and Brooks *(9)* found practically constant contents of sugar and acid-hydrolyzable polysaccharides at all temperatures essayed. During storage (or in transit) virtually unchanged sugar contents were also reported for peaches by Allen *(1)*, Matsumoto *(8)*, Culpepper and Caldwell *(5)*, and Putterill and Dreyer *(16)*.

In both the bruised and unbruised fruit, in contrast to the results of Haller and Harding *(6)*, titrable acidity (*i.e.*, total free acid content) decreases as the temperature increases (Table 1). However, the rate of change differs in the 2 treatments. Although unbruised specimens held at 70° F. differ little from those at 34° F., the bruised peach at 70° F. has lost a quarter of the total acidity present at 34° F. The maximum difference in free acid content

between bruised and unbruised fruit probably occurs in the 70°-95° F. range. At temperatures lower and higher than this range, this difference decreases.

It is noteworthy that the acid content of the unbruised peach is equal to that of the bruised one at 34° F. and again at 130° F. At the lower temperature, it can be readily imagined, metabolic changes are considerably retarded; hence an acid-metabolizing system is not effectively accelerated by bruising. On the other hand, the destruction of differential permeability (*i.e.*, selectivity) of the cellular plasmatic membranes at the higher temperature (130° F.) is obviously identical with the loss of selectivity due to physical destruction of the cells. Moreover, the heat can be imagined to accelerate metabolic changes in the fruit to such an extent that a bruising effect is not significant. A temperature of 130° F. is certainly a lethal one for the parenchyma cells of the fruit.

The generally reported sensitivity of free acid content to storage conditions *(4, 5, 8)* is matched by its sensitivity to bruising. Since, except for quinic acid *(2)*, the principal acids reported for peaches are citric and malic *(3, 7, 10, 11, 13, 14)*, which are functional members of the Krebs cycle, it is reasonable to expect their preferential utilization in respiration. The practicality of stimulating free acid disappearance by bruising or by temperature control, in order to improve the flavor of free-stone peaches, remains to be tested under commercial conditions.

SUMMARY

Bruised and unbruised free-stone peaches were held at different temperatures for 20 hours. The moisture content decreased somewhat with higher temperature. Although the sugar content in both bruised and unbruised peaches remained fairly constant (probably due to a rate of destruction almost equivalent to the rate of water loss), the content of free acid decreased with increasing temperature. The maximum difference in acid content between bruised and unbruised fruit probably occurs at temperatures between 70° and 95° F. Acid content in bruised peaches is equal to that of unbruised at 34° F. and at 130° F.

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THE INFLUENCE OF SALT UPON PANEL SCORES OF IRRADIATED AND UNIRRADIATED BEEF ROASTS[a, b]

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Salt is not commonly added to meat in taste panel evaluation. This practice has been based upon two factors. First, panel members differ greatly in the amount of seasoning they prefer, and thus the addition of salt during preparation may affect scores. However, this objection can be overcome by allowing each panel member to add salt to his own individual taste. Second, it is a common belief that salt may result in masking of some flavor components, thereby preventing critical evaluation. It has been suggested *(2, 9)* that the flavors so masked may be either desirable or undesirable. Thus, the addition of salt may actually prevent detection and evaluation of certain desirable flavors, but on the other hand, may cover up certain undesirable components, thereby resulting in an improvement of score.

It is well established *(1, 3, 4, 7)* that irradiation produces off-flavors in meat products. Thus, if the addition of salt could be used to achieve any significant improvement in flavor of irradiated meat products, it would be of considerable importance. This is more meaningful when one considers that salt is commonly added to meat items either in preparation or by the consumer just prior to eating. Therefore, it is important that the effect of adding salt upon the scores of irradiated meat be investigated.

Although experiments have been conducted to investigate the effects of salt on panel scores for vegetables *(10)* and upon cooking losses of certain beef cuts *(8)*, information is not available in the literature on the effects of added salt upon the panel scores of beef. Consequently, this study was designed to ascertain the effects upon panel ratings of allowing members to add salt to their individual taste.

EXPERIMENTAL

Preparation of meat. Six pairs of U.S. prime wholesale beef ribs were obtained from cattle all fed in the same manner. Seven days after slaughter, the *Longissimus dorsi* was stripped from the wholesale ribs and paired right and left roasts were cut to fit into a No. 2 can. Roasts were inserted in polymylar bags, which in turn were placed in the cans and roasted to an internal temperature of 71° C. in an electric oven held at 149° C. Following roasting, the meat was cooled slightly and the bags were heat sealed. Cans were then sealed under 28 inches of vacuum and cooled to 2° C.

[a] Journal Article 2167 Michigan Agricultural Experiment Station, East Lansing.
[b] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 825 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author(s). They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

Allotment and irradiation. Paired cans were coded and labeled, so one can of each pair could be used as the unirradiated control and the other one could be irradiated. The cans to be irradiated were packed into boxes and taken to Ann Arbor, Michigan, where they were irradiated in the Fission Products Laboratory, University of Michigan. This trip required about 1½ hours time by automobile. All samples were irradiated with a gamma source at a level of 2.79 megarads and returned to the Michigan State University Meats Laboratory. All cans were stored at 2° C. except while being irradiated and enroute.

Presentation to panel. Paired samples were heated for 40 minutes in a 149° C. oven. Roasts were cut into portions and presented by pairs to experienced panel members. Thus, each judge received one irradiated and one unirradiated sample. Each panel consisted of 20 judges, one-half of which were allowed to salt the meat to their individual taste while the remainder were asked to score the meat without the use of salt. The study was designed so that each judge was reversed on his next subsequent sitting, i.e., those allowed to add salt were given no salt at the next sitting. A total of 6 different panels were held involving 120 paired judgments on 240 samples made by 46 different judges. All judges except 6 were reversed at least once, with 23 serving on 2 panels, 16 on 4 panels and 1 on all 6 panels. Scoring was on the hedonic scale *(5, 6)*.

Statistical analysis. Correlation coefficients were calculated for each set of judges and found to be not significantly different from zero, indicating that the second score of a judge was not related to his first score. Since there was no correlation between judges' scores, an analysis of variance was carried out. As there was no interaction between panels and treatments, the sum of squares due to this interaction was combined with the sum of squares due to variation between judges' scores to form the final error term.

RESULTS

Acceptability ratings are summarized in Table 1. The mean score of all irradiated samples (4.95) was significantly lower ($P = <.01$) than the corresponding rating for all unirradiated control samples (7.39). This is in agreement with a number of studies *(1, 3, 4, 7)* in which irradiation has been shown to lower acceptability.

TABLE 1

Panel scores of irradiated and unirradiated beef roasts with and without added salt

Panel No.	1	2	3	4	5	6	Means
Irradiated + added salt	5.00	5.08	4.09	5.50	5.82	4.64	5.02
Irradiated without salt	5.08	4.36	5.36	4.00	5.18	5.27	4.87
Control + added salt	8.00	7.75	7.36	7.75	6.91	7.09	7.48
Control without salt	6.92	7.63	7.54	7.42	6.91	7.36	7.30
Means	6.25	6.20	6.09	6.17	6.20	6.09	6.17

Examination of the data in Table 1 shows all 6 mean panel scores were remarkably similar with a maximum range from 6.09 to 6.25. Thus, it is apparent that the reaction of each panel was alike. In considering the irradiated samples with added salt, the range was from 4.09 to 5.82, while the irradiated samples without salt had a range of 4.00 to 5.36. Upon comparing all irradiated samples with added salt to the unsalted samples, the over-all means were 5.02 and 4.87, respectively. A breakdown of the data by individual panels reveals that in panels 1, 3 and 6 the irradiated samples without salt had slightly higher mean scores than those with salt, wheras with panels 2, 4 and 5 the reverse was true.

With the paired unirradiated control roasts, the over-all acceptability means were 7.48 for those with added salt and 7.30 for those without salt. Examination of the data by individual panels shows those without salt were rated higher by panels 3 and 6 and were equal in panel 5, while those with added salt were preferred by panels 1, 2 and 4. This is in general agreement with the work of Whitacre *(10)*, in which no evidence was found that either addition or omission of salt in cooking vegetables enabled judges to detect small differences better in one case than another.

Statistical analysis (Table 2) showed that even though the difference between the irradiated and unirradiated samples was significant at the 1% level, there was no significant difference between the salted and unsalted sample averages regardless of whether they

TABLE 2
Summary of analysis of variance

Source of variation	Degrees of freedom	Mean square
Total	239	—
Panels	5	0.7
Irradiated versus unirradiated (I)	1	345.6*
Salt versus no salt (S)	1	1.1
Interaction (I × S)	1	1.7
Error term	231	3.3

* Highly significant

were irradiated or unirradiated. Furthermore, the interaction was not significant, showing that both irradiated and unirradiated samples with or without added salt were preferred equally.

DISCUSSION

Members of panels evaluating irradiated meats have frequently commented that salt and other seasoning would help to obscure the flavors produced by irradiation. However, examination of the data fails to substantiate this belief. Although the over-all differences were slightly higher for the samples to which salt was added, the differences did not approach significance. The fact that added salt was without influence on panel ratings is borne out by examination of the data by individual panels for both irradiated and unirradiated samples (Table 1). With the irradiated roasts, a comparison of acceptability ratings shows that the unsalted samples were preferred by 3 out of 6 panels, thus verifying the fact that the difference in scores was not significant. Acceptability ratings for the unirradiated control roasts without added salt were rated higher by 2 out of 6 panels and were equal once. Thus, it was shown that allowing panel members to add salt to samples was without measurable influence on acceptability ratings.

It is obvious from the results of this study that adding salt to either irradiated or unirradiated beef roasts was without effect on score. Thus, there is no apparent reason why salt should be used in scoring either unirradiated or irradiated beef roasts. As the addition of salt during preparation requires extra effort and adds another variable, there appears to be no sound basis for adding salt to obscure the irradiation flavor. This is apparent when it is considered that allowing each judge to add salt to his own individual taste did not appear to measurably alter his score as compared to the unsalted product.

Even though there was no obvious reason for adding salt to either control or irradiated beef roasts, this does not produce the possibility of salt masking certain desirable or undesirable flavors in other types of foods processed in a different manner. However, this study shows that added salt does not measurably improve the score of unirradiated controls or irradiated beef roasts.

SUMMARY

The effect of added salt upon panel scores of irradiated and unirradiated beef roasts was studied. Allowing panel members to salt meat to their own

individual taste did not significantly alter preference ratings for either the irradiated or unirradiated meat. Thus, the addition of salt did not mask the irradiation flavor or have any apparent influence on the flavor of the unirradiated meat. Consequently, there would appear to be little reason for using added salt in evaluation of either irradiated or unirradiated roasts.

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SOME STATISTICAL CONSIDERATIONS FOR SMALL SAMPLE EVALUATION IN TRIANGULAR TASTE TESTS

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A common problem with which taste panels are faced is that of distinguishing between two similar samples. The most widely used experimental designs for the solution of this problem are pair tests, duo-trio tests, triangle tests, multiple comparison tests, and their various modifications. All of the preceding tests have in common the fact that a decision is based on the proportion of correct judgments made by the panel members. In comparing the relative merits of the various experimental designs, apparently conflicting results have been obtained. Byer and Abrams *(2)* conclude that pair tests are superior to triangle tests. However, Harrison and Elder *(5)* call the triangle test "obviously more efficient" than the pair test. Gridgeman *(4)* concludes that " . . . pair tests and triangle tests are normally about equally powerful and appreciably superior to duo-trio tests." Hopkins and Gridgeman *(6)* have shown that, in consideration of their respective powers, "triangular tests have a statistical advantage over duo-trios and pairs." In certain situations, experimental evidence would seem to indicate a preference for multiple comparisons *(11, 12)*. The purpose of this paper is to consider a modification in the analysis of the triangular test, rather than to indicate the best experimental design (even if such were possible).

Although other than statistical considerations are important in most experimental situations, the statistical advantage of triangular taste tests is of sufficient merit to warrant further investigation of this technique. With this in mind, the authors have developed a statistical procedure for the evaluation of triangle test results which improves the analysis of these results, using as a criterion of improvement the powers of the conventional and modified test procedures.

Although the emphasis is primarily on the statistical aspects of this technique, it is recognized that the usual problems of coding bias, panel selection, fatigue, and other personal factors exist and must be dealt with by the experimenter. For discussions of these problems, the reader may consult the citations *(1, 7, 9, 10, 13)* contained in the reference list.

SOME THEORETICAL ASPECTS

Let us assume, for the purpose of this paper, that the primary concern rests in the question: "Is there a detectable difference between samples A and B?" The intensity *(3)* of such a difference has been omitted from consideration, not only to simplify the analysis of the test but also due to the fact that practical situations exist for which the intensity is not a factor.

For example, a certain commodity X contains 1% of an additive Y. It is desired to increase the percent of Y without substantially changing the flavor of X. If the flavor difference is detectable, the percent of Y will not be changed. However, if increasing the percent of Y to say 3% does not incur a detectable difference, the higher percent of Y may be used. In cases such as this, the intensity of the difference is of no consequence. The question is: Does a difference exist (regardless of degree)?

In a triangle taste test, wherein the panel attempts to determine which of 3 aliquots is the "odd" or different aliquot, one would expect to pick the "odd"

aliquot once in every 3 times on the average by merely guessing. In the notation of Gridgeman *(4)*, let us define the probability of correct discrimination as P_d and the probability of picking the "odd" aliquot by guessing as P_g equals 1/3. Then the proportion of correct judgments in the long run is, by the law of compound probability:

$$P = P_d + P_g (1 - P_d) \text{ or } P = (1 + 2P_d)/3.$$

Obviously, if there is no detectable difference in the samples, P_d is 0 and P is 1/3. However, if there is a readily detectable difference, P_d will be greater than 0 and hence P will be greater than 1/3. This suggests the null hypothesis that the probability of correct discrimination is zero, which is the common null hypothesis for sensory difference tests. An alternative hypothesis that P_d is greater than zero is then also suggested by the foregoing. Of course, in practice P_d is seldom if ever specifiable *a priori*. This poses no difficulty in the present situation, however, if we consider the alternative hypothesis specifying a set of values of P_d (any P_d greater than zero) rather than some single value. Clearly, the null hypothesis implies no detectable difference whereas the alternative hypothesis implies the opposite. Also, for the sake of simplicity, we shall assume equality of the P_d for all judges of a given flavor contrast. For testing this assumption, as well as for estimating P_d, the reader is referred to Hopkins and Gridgeman *(6)*. (The authors are presently considering more general alternative hypotheses wherein equality of P_d for all judges is not assumed.)

In terms of a statistical test procedure, the null and alternative hypotheses are specified by

$$H_o : P_d = 0 \text{ or } H_o : P = P_g = 1/3$$

and

$$H_1 : P_d > 0 \text{ or } H_1 : P > P_g = 1/3$$

respectively. The alternative hypothesis is then a composite hypothesis, i.e. it is consistent for more than one value of P. For testing the above hypotheses, a one-tailed test should be used. In other words, the critical region (that set of results which leads to the rejection of the null hypothesis and implies the acceptance of the alternative hypothesis) consists of only large values of P ($P > 1/3$).

In any statistical test procedure, two main types of errors are to be considered. These two errors are termed the type I and type II errors, where the type I error is the error made in rejecting H_o when in fact it is true and the type II error is the error made in failing to reject the null hypothesis when in fact it is not true. The above errors are commonly referred to as the producer's risk and the consumer's risk, respectively, since an error of the first type usually results in rejecting an acceptable item and an error of the second type usually results in the consumer being exposed to a nonacceptable item. In taste testing, committing a type I error results essentially in a mistake by which the consumer is not adversely affected. However, committing a type II error may result in adverse consumer reaction. Thus, it is desirable to keep the probability of both errors small, but especially the probability of a type II error. Unfortunately, the type II error is frequently neglected in analyzing taste test results *(14)*.

The probability of committing a type I error is denoted by α and the probability of committing a type II error is denoted by β. For a given test procedure, the critical region is determined by specifying α. The optimum

test situation is one in which, for a specified α, β is a minimum. However, for a fixed sample size, α and β vary inversely. Therefore, choosing $\alpha = 0$ would make β a maximum and conversely. In experimental situations, however, an $\alpha = 0$ is an impractical restriction; consequently, an α of .05 or .01 is conventionally used *(8)*. Minimizing β is equivalent to maximizing $1 - \beta$, which is called the power of the test. In a test situation where the alternative hypothesis is consistent for more than one value of P, the power of the test varies with the particular value of P which is selected. In this case, we obtain the power function which gives the power of the test for the various values of P between 0 and 1. Of course, the values of P which are of interest in the triangle test are $P = 1/3$ (H_o) and $P > 1/3$ (H_1). Values of P less than 1/3 are not meaningful in this situation. The power function then appears to be one reasonable criterion for choosing between two test procedures.

Conventionally, the procedure for analysis of triangle test results uses the binomial expression:

$$(1.1) \qquad (P + Q)^n = \sum_{x=o}^{n} \binom{n}{x} P^x Q^{n-x}$$

where x is the number of correct determinations out of a total of n determinations, P (the probability of a correct determination) is specified by the null hypothesis and $Q = 1 - P$. For a specified α, the critical region is determined by solving the expression:

$$(1.2) \qquad \sum_{x=x_o}^{n} \binom{n}{x} P^x Q^{n-x} \leq \alpha$$

for x_o. In a given experiment, the null hypothesis is rejected whenever the number of successes is equal to or greater than x_o. Since the binomial is a discrete distribution (x assumes only integral values), (1.2) is expressed as an inequality rather than an equality. Unfortunately, unless n is sufficiently large, the term on the left side of (1.2) will vary from α by a great deal (see section on procedure). Although it may not be obvious without a numerical example, this in turn increases β and hence decreases the power of the test.

As a partial solution to this problem, the authors propose the use of the multinomial rather than the binomial distribution. Let us assume, for the sake of simplicity, that the experiment involves 3 replications or trials by each judge. (It should be realized that small-sample designs involving other groupings of judges and replications are not only possible but of considerable interest. Power considerations would be of importance in these cases also, as a means of choosing appropriate designs. However, the authors feel that consideration of these cases would tend to make the present paper too lengthy for the purpose intended.) There are then 4 possible results. A judge may make 3, 2, 1, or 0 correct decisions. Let:

x_1 be the number of judges making 3 correct decisions
x_2 be the number of judges making 2 correct decisions
x_3 be the number of judges making 1 correct decision
x_4 be the number of judges making 0 correct decisions

and let:

Π_1 be the probability of a judge making 3 correct decisions
Π_2 be the probability of a judge making 2 correct decisions
Π_3 be the probability of a judge making 1 correct decision
Π_4 be the probability of a judge making 0 correct decisions.

The Π_i values are then determined by the following considerations: The probability of a judge making 3, 2, 1, 0 correct decisions out of 3 possible correct decisions, when the probability of a correct decision under the null hypothesis is 1/3, is given by the binomial expressions:

$$\Pi_i = \text{probability } (r, n) = \binom{n}{r} P^r Q^{n-r}$$

where $r = 3, 2, 1, 0$; $n = 3$; $P = 1/3$; and $Q = 1 - P = 2/3$.

We then have:

$$\Pi_1 = \text{probability } (3, 3) = \binom{3}{3} (1/3)^3 (2/3)^0 = 1/27$$

$$\Pi_2 = \text{probability } (2, 3) = \binom{3}{2} (1/3)^2 (2/3)^1 = 6/27$$

$$\Pi_3 = \text{probability } (1, 3) = \binom{3}{1} (1/3)^1 (2/3)^2 = 12/27$$

$$\Pi_4 = \text{probability } (0, 3) = \binom{3}{0} (1/3)^0 (2/3)^3 = 8/27$$

The null hypothesis could then be written as:

$H_o : \Pi_1 = 1/27, \Pi_2 = 6/27, \Pi_3 = 12/27, \Pi_4 = 8/27$

but this is exactly equivalent to writing:

$H_o : P = 1/3$.

Hence the simpler form will be used along with $H_1 : P > 1/3$. The multinomial expression corresponding to (1.1) is then:

$$(1.3) \quad (\Pi_1 + \Pi_2 + \Pi_3 + \Pi_4)^k = \sum_{x_i} \frac{K!}{X_1! X_2! X_3! X_4!} \Pi_1^{x_1} \Pi_2^{x_2} \Pi_3^{x_3} \Pi_4^{x_4}$$

where k is the number of judges on the panel and $\Sigma x_i = k$. For a specified value of a, (1.3) may be summed over the values of x_i which tend to discredit the null hypothesis and such that the sum is less than or equal to a. The relatively greater power of this method will be illustrated in the section on procedure.

PROCEDURE

For illustration, let us use 4 judges and 3 replications. There are then 12 possibilities of a correct determination. If no readily detectable difference exists between the samples, approximately 4 correct decisions (1/3 of 12) would be expected. The reader should realize, however, that the proportion of correct judgments will be close to 1/3 only if the experiment is repeated a large number of times. In single experiments, the results will vary about 1/3 by a chance amount when there is no readily detectable difference. The hypotheses are:

$$H_o : P = 1/3 \text{ and } H_1 : P > 1/3.$$

Let us select an a of .05. This means that a certain set of results will be assumed to indicate a real difference between samples whenever the chance of such results is less than 5 in 100 under the null hypothesis. Under these conditions we shall, of course, expect to be wrong 5 times out of 100 in the long run. The two forms of analysis follow:

Binomial:

Here we have:

$$\sum_{x=o}^{n} \binom{n}{x} P^x Q^{n-x} = \sum_{x=o}^{12} \binom{12}{x} (1/3)^x (2/3)^{12-x} \tag{1.4}$$

Then (1.2) becomes:

$$\sum_{x=x_o}^{12} \binom{12}{x} (1/3)^x (2/3)^{12-x} \leqq .05 \tag{1.5}$$

which is found to be consistent for $x_o = 8$ by consulting the binomial tables *(15)*. Therefore in this test, the null hypothesis would be rejected whenever 8 or more correct determinations were made. It should be pointed out, however, that the left side of (1.5) is actually equal to .019 for $x_o = 8$.

Multinomial:

The terms of the multinomial are given by:

$$\sum_{x_i} \frac{4!}{X_1! X_2! X_3! X_4!} (1/27)^{x_1} (6/27)^{x_2} (12/27)^{x_3} (8/27)^{x_4} \tag{1.6}$$

These are arranged by ordering the number of successes (0–12) and the terms corresponding to each number of correct determinations. (Actually then, the first stage ordering of these terms is a binomial ordering and the multinomial ordering is the second stage. However, since the improvement results from the multinomial break-down and ordering, the whole procedure is termed multinomial.) The reader will notice that for each number of correct determinations there may be several multinomial terms. These terms, each corresponding to the same number of correct determinations, are then ordered by considering the probability of their occurrence under H_o and H_1, i.e., the terms least likely under H_o are placed nearest the rejection region. In ordering the multinomial terms, several possibilities exist, but the method used herein is felt to be the most practical since the probabilities of terms in the rejection region consistently increase with increasing values of P. This illustrates the practical utility of the multinomial in contrast to the binomial. The binomial considers only the number of correct determinations, whereas the multinomial also considers the ways in which a given number of correct determinations could originate.

For the case considered here, the inequality:

$$\sum_{x_i} \frac{4!}{X_1! X_2! X_3! X_4!} (1/27)^{x_1} (6/27)^{x_2} (12/27)^{x_3} (8/27)^{x_4} \leqq .05 \tag{1.7}$$

is satisfied for 7 correct decisions or more providing that the 7 correct decisions are a result of:

$$\begin{gathered} x_1 = 2, x_2 = 0, x_3 = 1, x_4 = 1 \\ x_1 = 1, x_2 = 2, x_3 = 0, x_4 = 1 \\ \text{and} \\ X_1 = 0, X_2 = 3, X_3 = 1, X_4 = 0 \\ \textit{or} \\ X_1 = 1, X_2 = 1, X_3 = 2, X_4 = 0 \end{gathered} \tag{1.8}$$

A problem, unique (among the results included) to 12 possible correct decisions, is encountered in this case. Both of the last two results have the same probability of occurrence; but, if both are included in the rejection region (1.7) becomes approximately .066 and the inequality is not satisfied. Two possible solutions are to include only one of the results and to include neither of the results. Including only one, (1.7) is approximately .047. Including neither, (1.7) is approximately .027. However, in both cases the resultant power is greater than under the binomial. In the binomial case, the corresponding expression is approximately .019. If one of these two results were obtained experimentally, an objective decision could be made based on the combination of α and β which was most advantageous to the experimenter. For example, the maximum α and minimum β would be obtained if both results were included and the converse would be true if neither were included. For the purposes of constructing the multinomial power function, one of the two results was included in the rejection region.

Tables 1 and 2 give the results which lead to a rejection of the null hypothesis for an α of .05 and .01, respectively, when 1, 2, 3, 4, 5, or 6 judges and 3 replications are used. For example, the results derived above are listed in Table 1 under 4 judges. There we find that 8 to 12 correct decisions and the additional results listed in (1.8) lead to a rejection of the null hypothesis for an α of .05.

TABLE 1

.05 Critical regions for multinomial triangular taste tests—1 through 6 judges; 3 replicates per judge

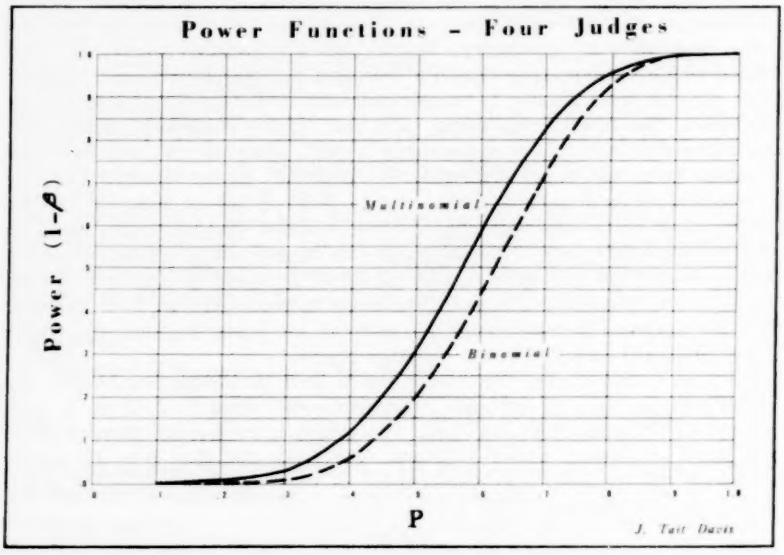
Number of judges:	1	2	3	4	5	6
Critical regions:	3 correct	5–6 correct	6–9 correct	8–12 correct and	9–15 correct and	10–18 correct and
				$X_1\ X_2\ X_3\ X_4$	$X_1\ X_2\ X_3\ X_4$	$X_1\ X_2\ X_3\ X_4$
				2 0 1 1	2 1 0 2	3 0 0 3
				1 2 0 1	2 0 2 1	1 3 0 2
				and	0 4 0 1	2 1 1 2
				0 3 1 0		2 0 3 1
				or		
				1 1 2 0		

TABLE 2

.01 Critical regions for multinomial triangular taste tests—1 through 6 judges; 3 replications per judge

Number of judges:	1	2	3	4	5	6
Critical regions:	*	6 correct	7–9 correct and	9–12 correct and	10–15 correct and	12–18 correct and
			$X_1\ X_2\ X_3\ X_4$	$X_1\ X_2\ X_3\ X_4$	$X_1\ X_2\ X_3\ X_4$	$X_1\ X_2\ X_3\ X_4$
			2 0 0 1	2 1 0 1	3 0 0 2	3 1 0 2
				2 0 2 0	2 0 3 0	3 0 2 1
				0 4 0 0		1 4 0 1
						0 5 1 0
						2 1 3 0
						2 2 1 1

* No rejection possible at .01.


Power Functions - Four Judges
Power (1-β)
Multinomial
Binomial
P
J. Tait Davis

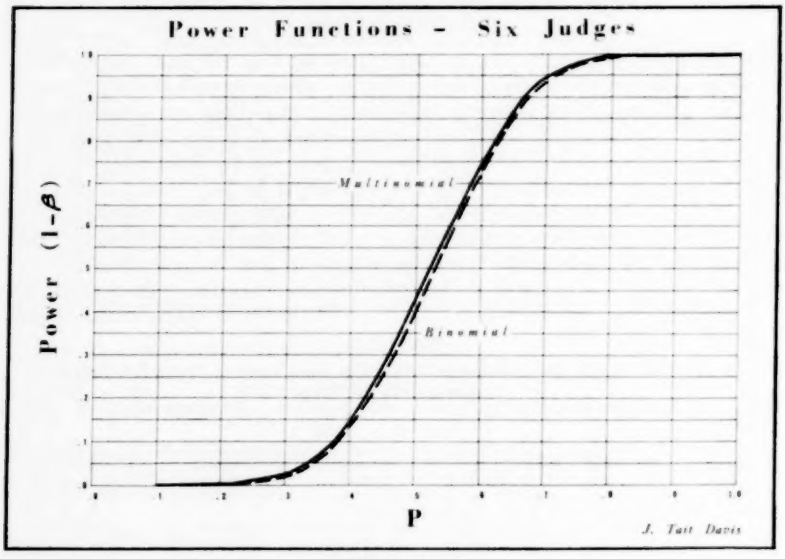

Power Functions - Six Judges
Power (1-β)
Multinomial
Binomial
P
J. Tait Davis

Figure 1

POWER OF THE TESTS

The power curves for the binomial and multinomial are given in Figure 1, for an α of .05 and for 4 and 6 judges. There, the power of the tests $(1 - \beta)$ is plotted against the various values of P. Since β is the probability of not rejecting H_o, when in fact some value of P consistent with H_1 is true, $1 - \beta$ may be arrived at by summing, for the various values of P, the probabilities of the results which fall in the critical region (See Tables 1 and 2). This has been done for values of P less than 1/3 also, in order to complete the power function. As pointed out previously, however, these values of P have no interpretation for the test in question. As evidenced by Figure 1, the power of the multinomial is consistently greater than that of the binomial for the two cases cited. (Similar results follow for an α of .01). For more than 6 judges and 3 replications, the two power curves practically coincide. For these situations then, there is little advantage in using the multinomial rather than the binomial from the point of view of power. However, there is a sufficient number of small sample cases to warrant the application of the results derived from consideration of the multinomial.

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A REVISED TABLE OF THE P_h FUNCTION OF BALL AND OLSON

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Ball and Olson *(1)* have developed a new formula method for calculating canning processes which has many important advantages over the older methods. It makes use of the very simple equation (equation 14.13a of their book),

$$p = \frac{Z}{F}(fP_h + cP_c), \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where p is the percentage lethal heat necessary to sterilize,

F is the number of minutes necessary to destroy organism at 250° F,

f is the usual parameter representing the slope of the heating curve,

c is the corresponding parameter representing the slope of the cooling curve,

Z is antilog $(T_1 - 220)/z$,

T_1 is the retort temperature,

z is the slope of the thermal death time curve,

P_h and P_c are functions of g and z which have been tabulated, and

g is the temperature difference between the critical point in the can and the heating mdium at the end of the heating phase.

The term fP_h is a measure of the lethal value of the heating phase and cP_c is the corresponding measure for the cooling phase.

Ball and Olson used graphical methods for determining the values of P_h and P_c quoted in their Tables. An analytical expression can be obtained for P_h which permits greater accuracy of calculation than is generally feasible by graphical methods. This has been used in preparing the Table presented here.

Alternative method of calculating P_h. If the heating curve is linear when plotted logarithmically in the usual way, it follows from equation (12.18) of Ball and Olson *(1)* and from their definition of P_h that

$$P_h = \frac{100 \times 10^{-80/z}}{2.3026}\left[\left(-\mathrm{Ei}(-2.3026g/z)\right) - \left(-\mathrm{Ei}(-2.3026jI/z)\right)\right], \quad (2)$$

where j is the lag factor expressed in the usual way and I is the initial temperature difference between the product and the heating medium. The second exponential integral in equation (2) is always negligible when the deviation of the heating curve from its asymptote (or tangent) is negligible at all temperatures above 200° F. or so. When this term is neglected, P_h becomes a function of g and z only and it is easily calculated with the aid of Tables of exponential integrals (2). Equations (1) and (2) are framed in terms of simple, unbroken heating curves but it is one of the great virtues of the new method that it is very easily adapted to deal with broken heating curves or other more complicated cases. These adaptations have been described by Ball and Olson *(1)* and they need not be discussed further here.

TABLE 1

Values of the Function $10^{30/s}P_h$

R/z	10	11	12	13	14	15	16	17	18	19	20	21
0.10	139.7	143.8	147.5	150.9	154.0	157.0	159.7	162.4	164.8	167.1	169.3	171.4
0.15	122.6	126.6	130.3	133.6	136.8	139.7	142.4	145.0	147.5	149.8	151.9	154.0
0.20	110.6	114.5	118.2	121.5	124.6	127.5	130.3	132.8	135.2	137.5	139.7	141.8
0.25	101.4	105.3	108.9	112.2	115.3	118.2	120.9	123.4	125.8	128.1	130.3	132.3
0.3	93.95	97.82	101.4	104.7	107.7	110.6	113.3	115.8	118.2	120.4	122.6	124.6
0.4	82.41	86.20	89.69	92.92	95.93	98.74	101.38	103.87	106.22	108.46	110.58	112.61
0.5	73.67	77.38	80.80	83.97	86.92	89.69	92.29	94.75	97.07	99.28	101.38	103.38
0.6	66.69	70.32	73.67	76.78	79.69	82.41	84.98	87.40	89.69	91.87	93.95	95.93
0.7	60.93	64.48	67.76	70.82	73.67	76.36	78.88	81.27	83.53	85.68	87.73	89.69
0.8	56.05	59.52	62.74	65.74	68.54	71.18	73.67	76.03	78.26	80.39	82.41	84.35
0.9	51.84	55.24	58.39	61.34	64.10	66.69	69.15	71.47	73.67	75.77	77.77	79.69
1.0	48.16	51.48	54.58	57.47	60.18	62.74	65.15	67.44	69.62	71.69	73.67	75.57
1.2	42.01	45.19	48.16	50.95	53.57	56.05	58.39	60.62	62.74	64.76	66.69	68.54
1.4	37.05	40.09	42.94	45.63	48.16	50.56	52.84	55.00	57.07	59.04	60.93	62.74
1.6	32.94	35.85	38.59	41.18	43.63	45.95	48.16	50.27	52.28	54.20	56.05	57.82
1.8	29.48	32.26	34.90	37.39	39.76	42.01	44.16	46.20	48.16	50.04	51.84	53.57
2.0	26.52	29.19	31.72	34.12	36.41	38.59	40.67	42.67	44.57	46.40	48.16	49.85
2.2	23.97	26.52	28.95	31.27	33.48	35.59	37.61	39.55	41.41	43.19	44.91	46.57
2.4	21.74	24.19	26.52	28.75	30.89	32.94	34.90	36.78	38.59	40.33	42.01	43.63
2.6	19.79	22.12	24.37	26.52	28.59	30.57	32.47	34.30	36.07	37.77	39.40	40.99
2.8	18.06	20.29	22.45	24.53	26.52	28.44	30.29	32.07	33.79	35.44	37.05	38.59
3.0	16.52	18.66	20.73	22.73	24.66	26.52	28.32	30.05	31.72	33.34	34.90	36.41
3.5	13.346	15.265	17.139	18.962	20.73	22.45	24.12	25.73	27.30	28.82	30.29	31.72
4.0	10.896	12.616	14.311	15.974	17.60	19.19	20.73	22.24	23.71	25.13	26.52	27.87
4.5	8.970	10.511	12.045	13.561	15.05	16.52	17.96	19.36	20.73	22.07	23.38	24.66
5.0	7.435	8.816	10.203	11.586	12.96	14.31	15.64	16.95	18.24	19.50	20.73	21.94
5.5	6.197	7.435	8.690	9.951	11.210	12.46	13.70	14.92	16.12	17.31	18.47	19.61
6.0	5.191	6.299	7.435	8.585	9.741	10.90	12.04	13.18	14.31	15.42	16.52	17.60
6.5	4.365	5.359	6.386	7.435	8.496	9.563	10.630	11.692	12.75	13.79	14.83	15.85
7.0	3.684	4.575	5.504	6.461	7.435	8.420	9.411	10.401	11.39	12.37	13.35	14.31
7.5	3.119	3.917	4.758	5.630	6.525	7.435	8.354	9.278	10.203	11.13	12.04	12.96
8.0	2.648	3.363	4.124	4.919	5.741	6.582	7.435	8.297	9.163	10.030	10.896	11.758
8.5	2.254	2.895	3.582	4.308	5.063	5.839	6.631	7.435	8.246	9.061	9.877	10.692
9.0	1.922	2.496	3.119	3.781	4.474	5.191	5.926	6.676	7.435	8.201	8.970	9.741
9.5	1.643	2.158	2.721	3.324	3.960	4.623	5.306	6.005	6.715	7.435	8.160	8.889
10.0	1.407	1.868	2.377	2.928	3.512	4.124	4.758	5.410	6.075	6.751	7.435	8.124
11.0	1.036	1.407	1.824	2.282	2.774	3.296	3.843	4.409	4.993	5.590	6.197	6.813
12.0	0.7678	1.065	1.407	1.788	2.203	2.648	3.119	3.612	4.124	4.651	5.191	5.741
13.0	0.5715	0.8104	1.0901	1.407	1.757	2.137	2.543	2.971	3.420	3.886	4.365	4.857
14.0	0.4272	0.6193	0.8477	1.1113	1.407	1.731	2.081	2.454	2.847	3.258	3.684	4.124
15.0	0.3205	0.4747	0.6619	0.8812	1.130	1.407	1.708	2.033	2.377	2.740	3.119	3.512
20.0	0.07948	0.1310	0.1999	0.2872	0.3934	0.5185	0.6620	0.8234	1.0019	1.1969	1.407	1.631
30.0	0.005563	0.01135	0.02069	0.03456	0.05390	0.07948	0.1120	0.1520	0.1999	0.2561	0.3205	0.3934
40.0	0.000429	0.001081	0.002350	0.004556	0.008078	0.01332	0.02069	0.03062	0.04347	0.05967	0.07948	0.1032

TABLE 1
(Continued)

R/z	22	23	24	25	30	35	40	45	50	55	60	70	80
0.1	173.4	175.3	177.1	178.9	186.8	193.4	199.2	204.3	208.8	212.9	216.7	223.4	229.1
0.15	156.0	157.9	159.7	161.5	169.3	175.9	181.7	186.8	191.3	195.4	199.2	205.8	211.6
0.2	143.8	145.6	147.5	149.2	157.0	163.6	169.3	174.4	178.9	183.0	186.8	193.4	199.2
0.25	134.3	136.2	138.0	139.7	147.5	154.0	159.7	164.8	169.3	173.4	177.1	183.8	189.5
0.3	126.6	128.5	130.3	132.0	139.7	146.3	151.9	157.0	161.5	165.6	169.3	175.9	181.7
0.4	114.54	116.40	118.17	119.88	127.5	134.0	139.7	144.7	149.2	153.3	157.0	163.6	169.3
0.5	105.30	107.13	108.89	110.58	118.2	124.6	130.3	135.2	139.7	143.8	147.5	154.0	159.7
0.6	97.82	99.64	101.38	103.06	110.6	117.0	122.6	127.5	132.0	136.0	139.7	146.3	151.9
0.7	91.57	93.36	95.09	96.75	104.21	110.6	116.1	121.1	125.5	129.5	133.2	139.7	145.4
0.8	86.20	87.98	89.69	91.34	98.74	105.06	110.6	115.5	119.9	123.9	127.5	134.0	139.7
0.9	81.52	83.28	84.98	86.61	93.95	100.23	105.7	110.6	115.0	118.9	122.6	129.1	134.7
1.0	77.38	79.12	80.80	82.41	89.69	95.93	101.4	106.2	110.6	114.5	118.2	124.6	130.3
1.2	70.32	72.03	73.67	75.26	82.41	88.56	93.95	98.74	103.06	106.98	110.58	117.0	122.6
1.4	64.48	66.15	67.76	69.32	76.35	82.41	87.73	92.47	96.75	100.64	104.21	110.6	116.1
1.6	59.52	61.16	62.74	64.26	71.18	77.16	82.41	87.10	91.34	95.19	98.74	105.06	110.6
1.8	55.24	56.84	58.39	59.89	66.69	72.58	77.77	82.41	86.61	90.43	93.95	100.23	105.7
2.0	51.48	53.06	54.58	56.05	62.74	68.54	73.67	78.26	82.41	86.20	89.69	95.93	101.38
2.2	48.16	49.70	51.19	52.63	59.21	64.94	70.00	74.54	78.66	82.41	85.87	92.06	97.48
2.4	45.19	46.70	48.16	49.58	56.05	61.69	66.69	71.18	75.26	78.98	82.41	88.56	93.95
2.6	42.51	43.99	45.43	46.82	53.18	58.74	63.68	68.12	72.16	75.85	79.26	85.36	90.71
2.8	40.09	41.54	42.94	44.31	50.56	56.05	60.93	65.32	69.32	72.98	76.36	82.41	87.73
3.0	37.88	39.30	40.67	42.01	48.16	53.57	58.39	62.74	66.69	70.32	73.67	79.69	84.98
3.5	33.11	34.46	35.77	37.05	42.94	48.16	52.84	57.07	60.93	64.48	67.76	73.67	78.88
4.0	29.19	30.47	31.72	32.94	38.59	43.63	48.16	52.28	56.05	59.52	62.74	68.54	73.67
4.5	25.91	27.13	28.32	29.48	34.90	39.76	44.16	48.16	51.84	55.24	58.39	64.10	69.15
5.0	23.12	24.28	25.41	26.52	31.72	36.41	40.67	44.57	48.16	51.48	54.58	60.18	65.15
5.5	20.73	21.83	22.91	23.97	28.95	33.48	37.61	41.41	44.91	48.16	51.19	56.70	61.60
6.0	18.66	19.71	20.73	21.74	26.52	30.89	34.90	38.59	42.01	45.19	48.16	53.57	58.39
6.5	16.85	17.85	18.82	19.79	24.37	28.59	32.47	36.07	39.40	42.51	45.43	50.74	55.49
7.0	15.26	16.21	17.14	18.06	22.45	26.52	30.29	33.79	37.05	40.09	42.94	48.16	52.84
7.5	13.86	14.76	15.64	16.52	20.73	24.66	28.32	31.72	34.90	37.88	40.67	45.80	50.40
8.0	12.62	13.47	14.31	15.15	19.19	22.98	26.52	29.83	32.94	35.85	38.59	43.63	48.16
8.5	11.51	12.31	13.12	13.91	17.79	21.45	24.88	28.11	31.14	33.99	36.67	41.62	46.09
9.0	10.511	11.28	12.04	12.81	16.52	20.05	23.38	26.52	29.48	32.26	34.90	39.76	44.16
9.5	9.619	10.35	11.08	11.80	15.36	18.77	22.00	25.06	27.94	30.67	33.25	38.03	42.36
10.0	8.816	9.510	10.20	10.90	14.31	17.60	20.73	23.71	26.52	29.19	31.72	36.41	40.67
11.0	7.435	8.061	8.690	9.320	12.460	15.52	18.47	21.29	23.97	26.52	28.95	33.48	37.61
12.0	6.299	6.865	7.435	8.009	10.896	13.75	16.52	19.19	21.74	24.19	26.52	30.89	34.90
13.0	5.359	5.869	6.386	6.909	9.563	12.22	14.83	17.35	19.79	22.13	24.37	28.59	32.47
14.0	4.575	5.035	5.504	5.979	8.420	10.896	13.35	15.74	18.06	20.29	22.45	26.52	30.29
15.0	3.917	4.333	4.758	5.191	7.435	9.741	12.04	14.31	16.52	18.66	20.73	24.66	28.32
20.0	1.868	2.117	2.377	2.648	4.124	5.741	7.435	9.163	10.896	12.616	14.311	17.60	20.73
30.0	0.4747	0.5641	0.6619	0.7678	1.407	2.203	3.119	4.124	5.191	6.299	7.435	9.741	12.04
40.0	0.1310	0.1632	0.2000	0.2412	0.5185	0.9107	1.407	1.991	2.648	3.363	4.124	5.741	7.435

A problem in the practical use of the table of P_h of Ball and Olson is that P_h varies rapidly with z in a way which makes it very difficult to interpolate accurately along horizontal lines in the Table i.e. between integral values of z. The function $10^{30/z}P_h$ is much more convenient in this respect and therefore the recalculated values have been tabulated in this form in Table I.

DISCUSSION

The factor $10^{30/z}$ included in the entries in Table 1 is, of course, the value of Z in equation (1) when the retort temperature is 250° F. If P_h and P_c are multiplied by this factor then Z should be replaced by $10^{-(250-T_1)/z}$.

A number of discrepancies were found between the P_h values quoted by Ball and Olson and those calculated from equation (2). In the parts of the Table which will be most often required in practice these discrepancies were mainly of the order which might be expected for errors in graphical integration and smoothing. Larger discrepancies were found at some of the extremes of the Table, particularly for very large g values which will very rarely be of practical interest. These probably arose from graphical extrapolation, which can be a hazardous procedure.

The values in Table *(1)* are quoted to four significant figures. This represents far greater accuracy than is usually warranted by the experimental data which must be used in process calculations. The uncertainties in experimental data are usually sufficient to make the third significant figure in a calculated lethal value very dubious and the second figure is generally uncertain.

Linear interpolation along horizontal lines in Table I is usually accurate to at least 0.2% for z not exceeding 25. Interpolation in the vertical columns may be more troublesome. In most parts of the Table, linear interpolation in the columns will usually be reliable to within 1% and this will generally be adequate. The use of an interpolation formula involving second differences, such as Bessel's formula with higher differences neglected, will generally give accuracy to within 0.2% except when $g > 15$. Accurate values for $g > 15$ seem unlikely to be required in practice except on very rare occasions and then it will probably be best to calculate them directly from equation (2).

Some values of z greater than 25 are included in Table I. They are unlikely to be required for ordinary process calculations but, following the precedent of Ball and Olson, they are included for the calculation of the destruction of thiamine or predicting the course of other reactions which have similar temperature coefficients.

The function P_c cannot be expressed by a simple analytical expression. Moreover an approximation to the form of the cooling curve is used by Ball and Olson and there is evidence *(3, 4, 5)* that it is not always a good approximation. Consequently it is desirable to investigate the factors affecting the form of cooling curves in more detail before attempting to recalculate P_c. Until this is done it will be desirable to have a method for making practical use of Table 1 together with Ball's approximation for the cooling phase. The simplest procedure is probably to make use of Ball's ρ function, which is the ratio of the lethal value of the heating phase to the total lethal value. When $f = c$,

$$\rho = \frac{P_h}{P_h + P_c} = \frac{10^{30/z}P_h}{10^{30/z}(P_h + P_c)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Consequently

$$p = \frac{10^{-(250-T_1)/z}}{F} \cdot \frac{f}{\rho} \cdot 10^{30/z} P_h \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

and ρ is easily calculated using Table 1 of this paper and Table 12.4 (Ball and Olson, *1*). When f is *not* equal to c, a better procedure will be to substitute for ρ in equation (4) the expression $\frac{f P_h}{f P_h + c P_c}$, using values of P_h and P_c taken from Tables 14.3 and 14.4 of Ball and Olson to evaluate this expression.

Acknowledgment

The author is indebted to Miss Joan Hayhurst for the computation of Table 1.

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THE *IN VITRO* DIGESTIBILITY AND NUTRITIONAL QUALITY OF DEHYDRATED BEEF, FISH AND BEANS[a]

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Interest in the dehydration of foods has increased steadily in recent years. Dehydration is not only an effective means of preserving foods but also provides significant savings in shipping space, weight and refrigeration. Suitable methods have now been developed for the commercial dehydration of meat, fish *(3, 4, 5)*, and bean *(2, 10)*. Recently, Sheffner, Adachi, and Spector *(13)* published an *in vitro* method (PDR index) which accurately measures the net utilization value (biological value × digestibility) of food proteins. The new procedure not only considers changes in total amino acid content but also the enzymatic availability of the component amino acids and has been successfully applied to the nutritional evaluation of heat processed and irradiated proteins *(12, 13)*. The purpose of the present investigation was to determine by means of the PDR index the nature and extent of the nutritive changes in the proteins of beef, fish and beans which result from dehydration.

EXPERIMENTAL

The beef[c] selected for this study was U. S. Good grade sirloin butt steak. Steaks were sliced one-half inch thick and were about 4 ounces in weight. The steaks were frozen and freeze-dehydrated at a plate temperature of 110° F. for 24 or 48 hours depending upon the desired moisture content of the final product. The steaks were vacuum packed in cans or flexible packages with and without an in-package desiccant (calcium oxide). Dehydrated steaks were rehydrated by soaking for 30 minutes at room temperature. The steaks were pan-fried without added fat at 375° F. for a total time of 2½ minutes, the steaks being turned every one-half minute.

Haddock[c] caught in the mid-Atlantic Ocean during the late summer months was used in this investigation. The fresh haddock was on ice 6 days before it was filleted. Fillets were laid in 10-pound blocks, frozen and sawed into sticks. Frozen fish sticks were freeze-dehydrated at a plate temperature of 100.4° F. and a pressure of 200-250 μ for 28-29 hours. The dehydrated sticks were hermetically sealed under vacuum in cans. The fish sticks were rehydrated by soaking in water one minute at room temperature. Cooked fish was obtained by frying the fish for one minute in deep fat heated to 375° F.

Navy (Michigan pea variety), red kidney, and baby lima (Birdseye brand) beans[d] were selected for this experiment. The navy beans were washed and soaked overnight. The soaked beans were then screened to remove those that had not swelled. Pre-cooking

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[c] Beef and haddock samples were prepared by James Blair, Animal Products Branch, Quartermaster Food and Container Institute for the Armed Forces.

[d] Beans were selected and prepared by technologists of Plant Products Branch, Quartermaster Food and Container Institute for the Armed Forces.

was done in a pressure retort at 245° F. for 20 minutes. The beans were frozen and dehydrated for one and one-quarter hours in a Proctor and Schwartz through-air-flow tray dehydrator at 170° F. utilizing low humidity. Red kidney beans were washed and soaked overnight, precooked at 245° F. in a steam-pressure cooker for 15 minutes, frozen, and dehydrated for six and one-half hours at 130° F. Fresh frozen lima beans were precooked by simmering at 212° F. for 18 minutes. The beans were then treated with 0.05% sulfite solution for one minute, drained, frozen, and dehydrated at 160° F. for 3 hours. Procedures for preparation of enzyme digests, amino acid analyses, and calculation of the PDR index were previously reported *(14)*.

RESULTS AND DISCUSSION

Table 1 presents the pepsin digest-residue (PDR) indexes of beef samples subjected to one or several of the following treatments: dehydration, rehydration, cooking, and storage. The results indicated that dehydration, rehydration, and cooking (fresh or rehydrated) did not change the nutritional

TABLE 1

The pepsin digest-residue (PDR) amino acid index values of fresh, dehydrated, cooked ,and stored beef

Treatment	PDR Index
None (frozen fresh)	76
Fresh cooked	77
Dehydrated (2% moisture)	77
Rehydrated	78
Rehydrated cooked	76
Frozen fresh, stored 12 mo., −20° F.	68
Dehydrated (2% moisture), stored 12 mo., −20° F., flexible package	81
Dehydrated (8% moisture), stored 12 mo., 70° F., flexible package	76
Dehydrated (2% moisture), stored 12 mo., 100° F., canned	80
Dehydrated (8% moisture), stored 12 mo., 100° F., canned	76

quality of the beef. Storage of raw beef at — 20° F. produced a 10% decrease in nutritional quality, due primarily to destruction of small amounts of cystine, isoleucine, leucine, lysine, tryptophan, tyrosine, and valine; the release of amino acids by pepsin digestion was not impaired. Beef steaks dehydrated to 2% moisture had slightly higher PDR index values after storage for 12 months either at — 20° F. in flexible containers or at 100° F. in cans. If the beef steaks were dehydrated initially to only 8% moisture and then stored at 70° F. in flexible packages or at 100° F. in cans they did not show the increased nutritional values; however, their values were equivalent to that of fresh beef.

The PDR index values of fresh, processed, and stored haddock are presented in Table 2. It is apparent that dehydration, rehydration, and storage did not affect the nutritional quality of haddock. For fresh and dehydrated haddock PDR index values of 78 were obtained. This checks closely with a net protein utilization value of 74 obtained for vacuum-dried haddock by the nitrogen balance method *(6)*. Under our conditions, cooking significantly increased the nutritive value of fresh and rehydrated haddock. Dehydrated haddock retains its nutritive value during storage even at 100° F. The essential amino acid composition of haddock was found to be in close agreement with that reported by Proctor and Lahiry *(11)* and Neilands *et al. (9)*.

TABLE 2

The PDR index values of fresh, dehydrated, cooked, and stored haddock

Treatment	PDR Index
None (frozen fresh)	78
Fresh cooked	86
Dehydrated	78
Rehydrated	76
Rehydrated cooked	83
Frozen fresh, stored 12 mo., −20° F.	78
Dehydrated, stored 12 mo., −20° F.	80
Dehydrated, stored 12 mo., 40° F.	77
Dehydrated, stored 12 mo., 100° F.	78

The effect of cooking upon the PDR indexes of kidney, lima, and navy beans is indicated in Table 3. The PDR index value of 75 for cooked lima beans is higher than the net utilization value of 58 reported by Metta and Johnson *(7)*. Although this difference can be accounted for by variations in conditions (freshness, maturity), methods of preparation, and variety of bean, the extremely low net utilization value of 34 which Metta and Johnson found for raw lima beans is probably due to the growth inhibitors present in many legumes. The PDR index has been shown to predict the net utilization value of this type of product only if the "toxic" factors are inactivated by mild heat treatment *(12)*. The results indicate, therefore, that the net utilization values of cooked kidney, lima, and navy bean proteins approximate the values of 69 to 72 obtained for soybean *(8, 12)* and are almost as good as those for beef protein. Everson and Heckert *(1)* also found that the biological value of heated lima beans compared closely with that of soybeans.

Dehydration of the cooked beans did not produce significant changes in the PDR index values (Table 3). In addition, chili con carne, a combination item containing precooked dehydrated kidney beans as well as similarly treated meat had a PDR value of 77 which is equivalent to that of good quality beef alone.

TABLE 3

Effect of cooking and dehydration upon the PDR index values of kidney, lima, and navy beans

Product	Treatment	PDR Index
Kidney bean	None	69
Kidney bean	Precooked	68
Kidney bean	Precooked, dehydrated	70
Lima bean	None	74
Lima bean	Precooked	75
Lima bean	Precooked, dehydrated	74
Navy bean	None	71
Navy bean	Precooked	70
Navy bean	Precooked, dehydrated	70
Chili con carne [1]	Precooked, dehydrated	77

[1] Composition: 58.4% precooked, dehydrated red kidney beans; 25.3% precooked, freeze-dried hamburger; 6.68% tomato solids; 3.9% beef soup and gravy base; 2.97% chili powder; 2.23% sodium chloride; and 0.03% garlic powder.

The effect of heating upon the enzymatic release of amino acids from lima beans is presented in Table 4. Data show that in general there is no change in the quantity of amino acids released by pepsin; however, following cooking of the beans there was a considerable increase in the amounts of amino acids released by the pepsin plus pancreatin treatment. Similar results were obtained with kidney beans. However, the quantity of amino acids released by pepsin plus pancreatin digestion of cooked lima beans was decreased by subsequent dehydration of the beans. Although it has been shown that the degree of digestibility with pancreatic enzymes *per se* is not correlated with the nutritional quality of a protein, the findings indicate that the

TABLE 4

Effect of cooking and dehydration upon the enzymatic release of amino acids from lima beans

Amino acid	Pepsin			Pepsin plus pancreatin		
	Unprocessed	Precooked	Precooked dehydrated	Unprocessed	Precooked	Precooked dehydrated
	% liberation			% liberation		
Histidine	8.1	6.5	6.7	16.2	39.0	34.2
Lysine	2.5	2.0	2.0	16.2	44.5	28.5
Methionine	19.0	17.6	15.8	35.4	47.6	36.5
Cystine	3.5	1.7	2.0	0.0	1.1	0.0
Phenylalanine	15.8	19.8	17.9	33.4	50.3	46.2
Tyrosine	8.6	8.8	8.8	27.5	52.5	46.9
Leucine	29.9	39.6	33.8	39.5	65.2	51.2
Isoleucine	31.1	37.2	35.8	37.0	56.2	53.4
Valine	15.0	12.2	12.4	24.6	35.8	30.6
Threonine	38.6	39.6	34.6	46.6	62.2	53.2
Tryptophan	15.2	16.3	13.1	21.5	35.1	26.3

dehydration process is causing structural changes in the bean proteins. Although these changes do not reflect a decrease in the protein quality of the present product, they suggest that any further increase in severity of the dehydration process with regard to time or temperature will affect the availability of the amino acids during digestion.

The total essential amino acid compositions of kidney, lima, and navy beans were found to be similar (Table 5). Examination of the essential amino acid pattern furthermore revealed that the bean proteins were deficient only in the sulfur amino acids, methionine and cystine. In this respect, lima beans contained more of cystine which in some measure accounts for the higher PDR values of lima beans. In addition, the average essential amino acid content of lima bean protein is slightly greater than that of the other two types of beans.

A comparison of the essential amino acid patterns of kidney and navy beans indicates that the pattern in kidney protein more nearly approaches that of the standard reference protein, whole egg. However, the protein in the kidney beans used in this study was considerably less susceptible to pepsin digestion than was the protein of the navy beans; consequently, the two factors balanced out and the PDR values for the two types of beans were very similar.

TABLE 5

Total[1] essential amino acid composition of unprocessed, precooked, and dehydrated kidney, lima, and navy beans

Amino acid	Unprocessed			Precooked			Precooked dehydrated		
	Kidney	Lima	Navy	Kidney	Lima	Navy	Kidney	Lima	Navy
Histidine	30.9	33.0	30.4	29.5	32.8	29.5	29.8	33.4	29.7
Lysine	73.4	70.0	71.5	70.0	71.6	70.3	74.1	71.9	73.1
Methionine	10.0	9.6	8.8	9.7	10.2	9.0	8.8	10.3	9.6
Cystine	8.8	13.6	9.0	8.2	13.1	8.6	7.8	11.1	8.0
Phenylalanine	56.6	53.7	52.0	57.0	56.5	53.8	56.0	55.8	52.2
Tyrosine	32.1	33.5	32.9	34.1	33.0	33.8	34.0	32.6	34.1
Leucine	86.4	90.3	81.2	86.8	85.9	81.1	87.0	92.7	80.2
Isoleucine	47.9	53.4	46.5	47.0	49.7	45.0	48.7	48.2	43.6
Valine	55.1	57.6	54.4	53.5	57.8	53.2	55.0	58.5	53.4
Threonine	44.8	47.0	52.2	44.2	52.5	52.4	42.4	54.8	52.7
Tryptophan	14.1	18.5	16.5	13.9	18.5	17.0	13.3	18.3	14.5

[1] Expressed as mg./g. of protein ($N \times 6.25$). Acid and alkaline hydrolysates were prepared as previously described *(13)*.

With respect to the destruction of amino acids, cooking, and dehydration were not found to have an appreciable effect upon the total essential amino acid content of any of the beans studied.

SUMMARY

Nutritional values are reported for dehydrated fish and meat, and precooked, dehydrated beans as measured by the PDR index. The results indicate that dehydration and cooking of haddock and beef, and precooking and dehydration of lima, kidney, and navy beans do not decrease the nutritional value of the proteins in these products. Dehydrated beef and haddock retained their nutritional quality on storage at 100° F. as well as or better than the frozen fresh products. The nutritional quality of kidney, lima, and navy bean proteins approximated that of soybean protein and was nearly as high as that of beef and fish.

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BUCKWHEAT AS A SUPPLEMENT TO ALL-VEGETABLE PROTEIN DIETS

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Successful all-vegetable rations for poultry feeding employing locally available feedstuffs have been previously developed for use in Central America *(6, 7)*. With this experience and other studies of the value of plants which grow well in the area, INCAP Mixture 8 (V M 8) a vegetable mixture for human consumption has been developed from local foods and tested *(1, 5)*. This formula has been shown to be effective for the treatment, and suitable for the prevention, of a form of severe protein malnutrition in children known as kwashiorkor which is common in Central America as in many other technically underdeveloped areas of the world.

In order to improve the commercial practicability of this V M 8 formula for human feeding and also to give greater flexibility to the formulation of poultry rations from locally grown plants, the use of buckwheat *(Fagopyrum esculentum)* as a source of carbohydrates and quality protein in such mixtures has been explored. Buckwheat is of particular interest since it is easily cultivated and yields well in the area and has been shown to have a relatively high nutritive value *(9)*.

MATERIAL AND METHODS

Buckwheat *(Fagopyrum esculentum)* is reported to contain 11.2% of protein, 2.4% fat, 64.0% carbohydrates, 10.7% crude fiber, 1.7% ash and to have a lysine content of 0.689% *(2, 3)*. The sample selected for these studies was grown in the Guatemala City area (elevation 5,000 feet). It was tested (a) as a replacement for the carbohydrate base of the V M 8 mixture and (b) as a source of lysine, which is one of the limiting amino acids of all-vegetable protein mixtures for chicks *(8)*.

Straight run 3-day old New Hampshire chicks were employed. No more than 12 chicks were housed in each all-wire cage. Heat was thermostatically controlled as required by the age of the bird. All experimental animals received water and feed *ad libitum*, and were weighed individually each week for a 35-day period. The rations fed, number, growth and the efficiencies of feed utilization of the chicks are presented in Table 1.

RESULTS

Ground buckwheat was first tested as a substitute for corn and sorghum. In experiment 1, the buckwheat was fed to replace 0.0%, 33.3%, 66.6% and 100.0% of the ground corn of an all-vegetable protein diet containing sesame, cottonseed and corozo oil meals as the principal sources of proteins. The significant linear response in the growth of the chicks and increased efficiency of feed utilization to each increased percent of buckwheat except the first indicates that buckwheat was superior to corn in these rations.

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TABLE 1

Effect of ground buckwheat as a substitute for the carbohydrates of corn and sorghum and as a supplemental source of lysine in all-vegetable protein rations for baby chicks

	Experiment 1				Experiment 2			Experiment 3		Experiment 4				
	1	2	3	4	1	2	3	1	2	1	2	3	4	5
Sesame oil meal	16.0	16.0	16.0	16.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Cottonseed oil meal	16.0	16.0	16.0	16.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Corozo oil meal	10.0	10.0	10.0	10.0	—	—	—	—	—	—	—	—	—	—
Ground corn	54.0	36.0	18.0	—	—	—	—	—	—	—	—	—	—	—
Ground sorghum	—	—	—	—	49.7	24.8	—	—	—	—	—	—	—	—
Ground whole buckwheat	—	18.0	36.0	54.0	—	24.9	49.7	49.3	49.7	49.7	49.5	49.3	47.7	45.7
Minerals	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cod liver oil	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
APF	0.7	0.7	0.7	0.7	—	—	—	—	—	—	—	—	—	—
Lysine	—	—	—	—	—	—	—	0.4	—	—	0.2	0.4	—	—
Blood meal	—	—	—	—	—	—	—	—	—	—	—	—	2.0	4.0
Kikuyu	—	—	—	—	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Average protein	20.0	20.0	20.0	20.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
No. of Birds, trial 1	12	12	12	12	24	24	24	24	24	24	24	24	24	24
No. of Birds, trial 2	24	24	24	24										
Av. final Wt. trial 1 g.	200	260	309	355	215	294	352	324	322	334	304	352	355	351
Av. final Wt. trial 2 g.	186	258	333	390										
Effic. Feed Util. trial 1[1]	3.21	3.86	2.93	2.29	2.83	2.58	2.66	2.58	2.69	2.58	2.79	2.61	2.67	2.63
Effic. Feed Util. trial 2	2.98	2.98	—	—										
Significance	Linear component highly significant				LSD 0.05 = 32			not significant		LSD 0.05 = 36				

[1] Grams of feed to produce 1 g. gain in weight.

In experiment 2, buckwheat was fed to replace the ground sorghum of an all-vegetable protein mixture containing sesame and cottonseed meal as the principal sources of protein (V M 8). The diets fed, number and growth rate of the chicks and efficiencies of feed utilization are presented in Table 1. It is obvious from the data that buckwheat was also superior to sorghum in the V M 8 type formula.

Results of experiments 1 and 2 were believed to indicate that the improvement observed in the growth of chicks fed buckwheat instead of corn or sorghum was due to its higher lysine content. To determine whether adding lysine would produce a further increase in growth, 0.4% was added to the vegetable ration containing buckwheat instead of corn. No additional effect of lysine on growth was observed. As may be seen in the table, the addition of the lysine had no apparent effect on chick growth. The lack of any supplemental value of lysine in this diet was tested again in experiment 4. In this trial, the ration containing the buckwheat was supplemented with 2 levels of (a) l-lysine and (b) a dried blood meal which was added as a natural source of lysine *(8)*. Data in the table show that again there was no apparent benefit from the addition of l-lysine or dried blood meal as shown by chick growth and efficiency of feed utilization.

DISCUSSION

All-vegetable protein rations for baby chicks are usually deficient in lysine *(8)*. The data of these trials indicate that when ground buckwheat replaced corn or sorghum in a simplified all-vegetable protein formula (INCAP V M 8), the lysine requirement of the chick was apparently satisfied. This was supported by the fact that the addition of two levels of l-lysine or of a dried blood meal, used as a natural source of lysine, did not have any further effect on chick growth or efficiency of feed utilization.

Results show that buckwheat could become an excellent substitute for the corn or sorghum in an all-vegetable protein mixture for baby chicks. From the standpoint of the ease of cultivation and yield, buckwheat should be more widely used in baby chick rations.

The sensitizing effect of raw buckwheat in several animal species is well known *(4, 10, 11)*; it produces an occasional eruption and irritation of the skin upon exposure to light. This need not interfere with its potential use in vegetable formulas for human consumption since these are invariably cooked and since buckwheat flour has been extensively used as a staple of human diets.

SUMMARY

Ground buckwheat, *Fagopyrum esculentum*, was found to be superior to corn and sorghum in an all-vegetable protein mixture containing sesame and cottonseed oil meals as the principal protein sources in rations for baby chicks. The data suggest that the improved growth of the chicks resulted from the additional lysine supplied by buckwheat in such a mixture.

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